IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Walker et al.
Serial No.	:	10/080,875
Filed	:	February 22, 2003
For	:	REGULATION OF INTRACELLULAR GLUCOCORTICOID CONCENTRATION
Examiner	:	Theodore J. Criares
Group Art Unit	:	1617

745 Fifth Avenue, New York, NY 10151

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

DECLARATION OF BRIAN R. WALKER AND JONATHAN R. SECKL

WE, BRIAN R. WALKER AND JONATHAN R. SECKL, declare and state that:

1. We are the named inventors on the above-captioned application ("the present application") and are familiar with it and its prosecution, including the claims, and the November 19, 2003 Office Action. It is our understanding that the pending claims read as follows:

> --14. A method for inhibiting reductase activity of 11-Beta-hydroxysteroid dehydrogenase 1 (11-Beta HSD1) in an animal in need thereof in neuronal tissue of the animal comprising administering to the animal an inhibitor of said reductase activity of 11-Beta HSD1 in an amount effective to so inhibit the reductase activity of 11-Beta HSD1.
>
> 15. A method for reducing intracellular glucocorticoid concentration in an animal in need thereof in neuronal tissue comprising inhibiting the reductase activity of 11-Beta-hydroxysteroid dehydrogenase 1 (11-Beta HSD1) in said tissue.
>
> 16. The method of claim 14 wherein the inhibitor is carbenoxolone or a pharmaceutically acceptable salt thereof.

17. The method of claim 15 wherein the inhibitor is carbenoxolone or a pharmaceutically acceptable salt thereof.--

2. More in particular, we are advised and therefore believe that in the November 18, 2003 Office Action, these claims were rejected under 35 U.S.C. §112 because the Examiner questions "compounds which inhibit the reductase activity of 11-Beta-hydroxysteroid dehydrogenase I in neural tissue" and enablement for such inhibitors beyond carbenoxolone.

3. We are also familiar with the present application and the concurrently-filed Communication forwarding Declaration, and that the arguments in that Communication are based on our assertions herein.

4. Furthermore we, Professor Jonathan R Seckl and Dr Brian R Walker, respectfully submit that we are experts in the field of 11β-hydroxysteroid dehydrogenases. Brief *curricula vitae* are attached as Appendices A and B. We have both been active researchers in this field for more than 10 years and, together and separately, have published more than 200 relevant primary articles in peer-reviewed journals and more than 80 review articles and contributions to books. We have obtained very substantial external research funding for our work in this area in open competition. We both lead research groups within the University of Edinburgh in which we supervise more than 40 full-time research staff who are investigating aspects of glucocorticoid biology, including 11β-hydroxysteroid dehydrogenases. We are both asked regularly to speak to the subject of 11β-hydroxysteroid dehydrogenase biology at national and international scientific meetings.

5. Accordingly, in view of our education, training and experience, we are considered by our peers to be experts in the field to which the present application pertains, and qualified to knowledgeably characterize the art to which the invention in the present application relates, and to speak as to the present application, and the invention claimed, including being qualified to present expert opinions about the present invention and literature in support of it, and documents cited against the present invention. Moreover, we respectfully submit that we are qualifed to state the knowledge in the art, and that which the skilled artisan would not have required any undue experimentation to practice, e.g., the enablement and the written description in the present application, and what the skilled artisan would have been taught, as well as what would have been obvious and nonobvious to the skilled artisan.

6. Thus, this Declaration is intended to assert the sufficiency of the enablement of the claimed subject matter of the present application (as of original filing of the parent application in August 1995), i.e., to respond to the rejections of the present application under 35 U.S.C. §112, first paragraph; which rejections we respectfully request be reconsidered and withdrawn in view of this Declaration and the attachments hereto. All documents cited herein are listed on a reference list that appears before the closing paragraph and our signatures. All documents cited herein are incorporated herein by reference, and a copy of those documents indicated in the following text as attached is included with this Declaration, to assist the Examiner in confirming our assertions and discussions herein. The Examiner is respectfully requested to consider and make of record documents cited herein.

7. With respect to the rejections under Sections 112, initially it is noted that the Figures provide doses of an inhibitor of the reductase activity of 11-Beta HSD1 from which the skilled artisan can make and use the claimed invention, without undue experimentation. Additionally, as to inhibitors of 11-Beta HSD1, the attached article by Monder and White, in Table IV at pages 196-198 provides a rather lengthy list of inhibitors of 11β-hydroxysteroid dehyrogenase, such that contrary to the Office Action, the skilled artisan understands compounds that "inhibit the reductase activity of 11-Beta-hydroxysteroid dehydrogenase I" and would readily understand how to use such compounds in the methods of the present invention without any undue experimentation.

8. Indeed, in addition to the lengthy list of inhibitors in Monder and White, we note that documents cited in the prosecution of the parent application, U.S. Application Serial Number 09/029,535, now U.S. Patent 6,521,267, also show inhibitors and modes of administration, such as Walker et al., "Carbenoxolone Increases Hepatic Insulin Sensitivity in Man: A Novel Role for 11-oxosteroid Reductase in Enhancing Glucocorticoid Receptor Activation," J. Clin. Endocrinology and Metabolism 80 (11): 3155-59 (1995). Thus, in the art, carbenoxolone and the lengthy list in Monder and White were known inhibitors. Gomez-Sanchez et al., "Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone," Am J Physiol 263 (6 Pt 1): E1125-E1130 (1992) showing that licorice, glycyrrhizic acid, and carbenoxolone were known inhibitors, as well as the infusion of glycyrrhizic acid and carbenoxolone into the lateral ventricle of the brain of the rat at doses less than that which has an effect when infused subcutaneously, produces hypertension, showing that such compounds were

administered subcutaneously, orally, and by infusion; *see also* Whorwood et al., "Licorice inhibits 11 beta-hydroxysteroid dehydrogenase messenger ribonucleic acid levels and potentiates glucocorticoid hormone action," Endocrinology 132 (6): 2287-92 (1993) (copy of Abstract attached). Even further still, Homma et al., "A Novel 11β-Hydroxsteroid Dehydrogenase Inhibitor Contained in Saiboku-To, a Herbal Remedy for Steroid-dependent Bronchial Asthma," J. Pharm Pharmacol 46:305-309 (1994) (copy attached), Zhang et al., "Inhibition of 11β-Hydroxysteroid Dehydrogenase Obtained from Guinea Pig Kidney by Furosemide, Naringenin and Some Other Compounds," J Steroid Biochem Molec Biol 49(1):81-85 (1994) (copy attached), and Lee et al., "Grapefruit juice and its flavenoids inhibit 11β-hydroxysteroid dehydrogenase," Clin Pharmacol Ther 59:62-71 (1996) (copy attached), evince even more inhibitors that can be administered in known ways (both in terms of doses and routes of administration), such as flavenoids, which "are sold in tablet form in health food stores and drug stores," and herbs or constituents of herbs. *See also* Morris et al., "Endogenous 11 beta-hydroxysteroid dehydrogenase inhibitors and their role in glucocorticoid Na+ retention and hypertension," Endocr Res 22(4):793-801 (1996) (progesterone metabolites as inhibitors, and progesterone is also a substance that can be administered – both in terms of doses and routes of administration - without undue experimentation).

9. Furthermore, attached as Appendix C are two pages of a presentation originally provided to the Patent Office during the October 2, 2001 Interview during the prosecution of U.S. Application Serial Number 09/029,535, now U.S. Patent 6,521,267, and which was provided to the present Examiner during the March 10, 2004 Interview. Appendix C depicts results obtained with various known compounds, including chenodeoxycholic acid and frusemide in addition to carbenoxolone, that inhibit 11B-reductase in intact primary neurons and adipocytes. Therefore, Appendix C provides additional known inhibitors that so inhibit the enzyme in amounts disclosed in the application, such that based upon the knowledge in the art and the disclosure in the application, the invention can be practiced by one of skill in the art without undue experimentation.

10. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the Section 112 rejections: The present application contains both a written description and enablement for the claimed methods, and, one skilled in the art, from the knowledge in the art and the teachings in the application, can practice the claimed methods, without any undue

experimentation, including without any undue experimentation in selecting a suitable inhibitor, and a dose therefore and a route of administration thereof.[a]

SUMMARY AND REQUEST FOR INTERVIEW

11. This declaration shows that the claimed subject matter is sufficiently described and enabled in the present application, and can be practiced without any undue experimentation. Accordingly, it is respectfully requested that the rejections of the November 18, 2003 Office Action be reconsidered and withdrawn. Moreover, we would welcome the opportunity to further explain any aspect of the present invention or this declaration to the Examiner, his SPE, and a Group 1600 Practice Specialist, in person. Therefore, if any issue remains as an impediment to allowance, we respectfully request a personal interview with the Examiner, his SPE, and a Group 1600 Practice Specialist, prior to issuance of any paper other than a Notice of Allowance; and, pursuant to this request the Examiner is also asked if he could please contact our representative, Mr. Thomas J. Kowalski, FROMMER LAWRENCE & HAUG LLP, 745 Fifth Avenue, New York, NY 10151, tel: 212-588-0800, fax: 212-588-0500, email: tkowalski@flhlaw.com, to arrange a mutually convenient time and manner for such an interview.

REFERENCES

12. References cited in this Declaration, and incorporated herein by reference, as shown in Appendix D, include:

Gomez-Sanchez et al., "Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone," **Am J Physiol** 263 (6 Pt 1): E1125-E1130 (1992).

Homma et al., "A Novel 11β-Hydroxsteroid Dehydrogenase Inhibitor Contained in Saiboku-To, a Herbal Remedy for Steroid-dependent Bronchial Asthma," **J. Pharm Pharmacol** 46:305-309 (1994).

Lee et al., "Grapefruit juice and its flavenoids inhibit 11β-hydroxysteroid dehydrogenase," **Clin Pharmacol Ther** 59:62-71 (1996).

Monder C, White PC. 11β-Hydroxysteroid dehydrogenase. **Vitamins and Hormones** 47: 187-271 (1993).

[a] In this regard, we are advised and therefore believe that a specification need only begin teaching where the prior art leaves off. Thus, the present application did not need to provide an exhaustive list in inhibitors, doses of inhibitors, and routes of administration.

Morris et al., "Endogenous 11 beta-hydroxysteroid dehydrogenase inhibitors and their role in glucocorticoid Na+ retention and hypertension," **Endocr Res** 22(4):793-801 (1996).

Walker BR, Connacher AA, Lindsay RM, Webb DJ, Edwards CRW. Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. **J.Clin.Endocrinol.Metab.** 80: 3155-3159 (1995).

Whorwood et al., "Licorice inhibits 11 beta-hydroxysteroid dehydrogenase messenger ribonucleic acid levels and potentiates glucocorticoid hormone action," **Endocrinology** 132 (6): 2287-92 (1993) (Abstract).

Zhang et al., "Inhibition of 11β-Hydroxysteroid Dehydrogenase Obtained from Guinea Pig Kidney by Furosemide, Naringenin and Some Other Compounds," **J Steroid Biochem Molec Biol** 49(1):81-85 (1994).

13. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Dated:____________________ By:________________________________
Professor Jonathan R. Seckl

Dated:____________________ By:________________________________
Dr. Brian R. Walker

CURRICULUM VITAE

Jonathan Robert **SECKL** BSc, MBBS, MRCP (UK), PhD, FRCPE, FMedSci

AWARDS, PRIZES AND FELLOWSHIPS

1978	Filliter Prize (1st in Pathology and Microbiology MB).
1980	Hons Viva (Medicine).
1980	Magrath Scholarship/Fellowes Gold Medal (Medicine MB).
1980	The Achison Exhibition (Medicine).
1984	Sir Jules Thorn Trust Research Fellowship.
1989	Wellcome Trust/Royal Society of Edinburgh Senior Clinical Research Fellowship.
1993	Wellcome Trust Senior Research Clinical Fellowship Renewal
1993	FRCP Edin
1994	Norage Pharmacia Prize (best paper on brain aging)
1998	Society for Endocrinology Medal
1999	Mortyn Jones Memorial Lecturer
1999	Fellowship, Academy of Medical Sciences

PRESENT APPOINTMENTS

1997	Moncrieff-Arnott Professor of Molecular Medicine, University of Edinburgh.
1995	Chairman, Molecular Medicine Centre, University of Edinburgh.
1989	Honorary Consultant Physician (Endocrinology), Western General Hospital.

PREVIOUS APPOINTMENTS

1996-97	Professor of Endocrinology, University of Edinburgh.
1993-96	Senior Lecturer in Medicine, University of Edinburgh.
1989-97	Wellcome Trust/Royal Society of Edinburgh Senior Clinical Research Fellow.
1987-92	Visiting Scientist, MRC Brain Metabolism Unit, Edinburgh.
1987-89	University of Edinburgh, Department of Medicine, Lecturer in Medicine
1984-87	Charing Cross and Westminster Medical School,Research Fellow Neuroendocrinology.

EDITORIAL BOARDS

Endocrinology (US); Steroids (US); Journal of Neuroendocrinology; Journal of Endocrinology

KEY RELEVANT PRIMARY PUBLICATIONS IN PEER-REVIEWED JOURNALS (OF 155)

Moisan M-P, **Seckl JR** and Edwards CRW (1990). 11ß-hydroxysteroid dehydrogenase mRNA expression and activity in rat hypothalamus, hippocampus and cortex. *Endocrinology* 127: 1450-1455.

Moisan M-P, **Seckl JR**, Brett LP, Monder C, Agarwal AK, White PC and Edwards CRW (1990). 11ß-hydroxysteroid dehydrogenase mRNA expression, bioactivity and immunoreact-ivity in rat cerebellum. *J Neuroendocrinol* 2: 853-858.

Moisan M-P, Edwards CRW and **Seckl JR** (1992). Ontogeny of 11ß-hydroxysteroid dehydrogenase bioactivity and messenger RNA expression in rat brain and kidney. *Endocrinology* 130: 400-404.

Moisan M-P, Edwards CRW and **Seckl JR** (1992). Differential promoter usage by the rat 11ß-hydroxysteroid dehydrogenase gene. *Molecular Endocrinol* 6: 1082-1087.

Seckl JR, French KL, O'Donnell D, Meaney MJ, Yates C and Fink G (1993). Glucocorticoid receptor gene expression is unaltered in hippocampal neurons in Alzheimer's disease. *Molec Brain Res* 18: 239-245.

Benediktsson R, Lindsay R, Noble J, **Seckl JR** and Edwards CRW (1993). Glucocorticoid exposure in utero: a new model for adult hypertension. *Lancet* 341: 339-341.

Edwards CRW, Benediktsson R, Lindsay R and **Seckl JR** (1993). Dysfunction of the placental glucocorticoid barrier: a link between fetal environment and adult hypertension? *Lancet* 341: 355-357.

Brown RW, Chapman, KE, Edwards CRW and **Seckl JR** (1993). Human placental 11ß-hydroxysteroid dehydrogenase: partial purification of and evidence for a distinct NAD-dependent isoform. *Endocrinology* 132: 2614-2621.

Low SC, Assaad SN, Rajan V, Chapman KE, Edwards CRW and **Seckl JR** (1993). Regulation of 11ß-hydroxysteroid dehydrogenase by sex steroids in vivo: further evidence for the existence of a second dehydrogenase in rat kidney. *J Endocrinol* 139: 27-35.

Leckie C, Chapman KE, Edwards CRW and **Seckl JR** (1995). LLC-PK$_1$ cells model 11ß-hydroxysteroid dehydrogenase type 2 regulation of glucocorticoid access to renal mineralocorticoid receptors. *Endocrinology* 136: 5561-5569.

Rajan V, Edwards CRW, **Seckl JR** (1996). 11ß-hydroxysteroid dehydrogenase in cultured hippocampal cells reactivates inert 11-dehydrocorticosterone, potentiating neurotoxicity *J Neuroscience* 16: 65-70.

Brown RW, Chapman KE, Edwards CRW and **Seckl JR** (1996). Purification of 11ß-hydroxysteroid dehydrogenase type 2 from human placenta. *Biochem J* 313: 997-1005

Brown RW, Kotolevtsev Y, Leckie C, Lindsay RS, Lyons V, Murad P, Mullins JJ, Chapman KE, Edwards CRW and **Seckl JR** (1996). Isolation and cloning of human placental 11ß-hydroxysteroid dehydrogenase-2 cDNA. *Biochem J* 313: 1007-1017

Brown RW, Diaz R, Robson AC, Kotolevtsev Y, Mullins JJ, Kaufman MH and **Seckl JR** (1996). The ontogeny of 11ß-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. *Endocrinol* 137: 794-797.

Voice M, **Seckl JR** and Chapman KE (1996). The sequence of 5'-flanking DNA from mouse 11ß-hydroxysteroid dehydrogenase type 1 and analysis of puative transcription factor binding sites. *Gene* 181: 233-235.

Lindsay RS, Lindsay RM, Edwards CRW and **Seckl JR** (1996). Inhibition of 11ß-hydroxysteroid dehydrogenase in pregnant rats and the programming of blood pressure in the offspring. *Hypertension* 27: 1200-1204.

Voice M, **Seckl JR**, Edwards CRW and Chapman KE (1996). 11ß-hydroxysteroid dehydrogenase type 1 expression in 2S-FAZA hepatoma cells is hormonally-regulated: a model for the study of hepatic corticosteroid metabolism. *Biochem J* 317: 621-625.

Waddell B, Benediktsson R and **Seckl JR.** (1996). 11ß-hydroxysteroid dehydrogenase type 2 in the rat corpus luteum: induction of mRNA expression and bioactivity coincident with luteal regression. *Endocrinology* 137: 5386-5391.

Lindsay RS, Lindsay RM, Waddell B and **Seckl JR** (1996). Programming of glucose tolerance in the rat: role of placental 11ß-hydroxysteroid dehydrogenase. *Diabetologia* 39: 1299-1305.

Rose KR, Stapleton G, Kieny M-P, Russell DW, Björkheim I, **Seckl JR**, Lathe R (1997). Cyp7b, a novel brain cytochrome P450, catalyses the synthesis of neurosteroids 7a-hydroxy DHEA and 7a-hydroxypregnenolone. *Proc Natl Acad Sci USA* 94: 4925-4930.

Kotolevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson PM, Best R, Brown R, Edwards CRW, **Seckl JR** and Mullins JJ (1998). 11ß-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid inducible responses and resist hyperglycaemia on obesity or stress. *Proc Natl Acad Sci USA* 94: 14924-14929.

Diaz R, Brown R, **Seckl JR** (1998). Ontogeny of mRNAs encoding glucocorticoid and mineralocorticoid receptors and 11ß-HSDs in prenatal rat brain development reveal complex control of glucocorticoid action. *J Neurosci* 18: 2570-2580.

Napolitano A, Voice M, Edwards CRW, **Seckl JR** and Chapman KE (1998). 11ß-hydroxysteroid dehydrogenase type 1 in adipocytes: expression is differentiation-dependent and hormonally-regulated. *J Steroid Biochem Molec Biol* 64: 251-260.

Waddell B, Benediktsson R, Brown R and **Seckl JR.** (1998). Tissue-specific mRNA expression of 11B-hydroxysteroid dehydrogenase types 1 and 2 and the glucocorticoid receptor within rat placenta suggest exquisite local control of glucocorticoid action. *Endocrinology* 139: 1517-1523.

Nyirenda M, Lindsay RS, Kenyon CJ, Burchell A and **Seckl JR** (1998). Glucocorticoid exposure in late gestation permanently programmes rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest* 101: 2174-2181.

Robson AC, Leckie C, **Seckl JR** and Holmes MC (1998). Expression of 11ß-hydroxysteroid dehydrogenase type 2 in the postnatal and adult rat brain. *Molec Brain Res* 61: 1-10.

Jamieson PM, Chapman KE, Walker BR and **Seckl JR** (1999). Interactions between oestradiol and glucocorticoid regulatory effects on liver-specific glucocorticoid-inducible genes: possible evidence for a role of hepatic 11 beta-hydroxysteroid dehydrogenase type 1. *J Endocrinol* 160: 103-109.

Jamieson PM, Chapman KE and **Seckl JR** (1999). Tissue- and temporal-specific regulation of 11ß-hydroxysteroid dehydrogenase type 1 by glucocorticoids in vivo. *J Steroid Biochem Molec Biol* 68: 245-250.

Kotelevtsev Y, Brown RW, Fleming S, Kenyon CJ, Edwards CRW, **Seckl JR** and Mullins JJ (1999). Hypertension in mice lacking 11ß-hydroxysteroid dehydrogenase type 2. *J Clin Invest* 103: 683-689.

Meaney MJ, Diorio J, Francis D, Weaver S, Yau JLW, Chapman KE, **Seckl JR** (2000). Postnatal handling increases the expression of cAMP-inducible transcription factors in the rat hippocampus: The effects of thyroid hormones and serotonin. *J Neurosci* 20: 3926-35.

Welberg LAM, **Seckl JR** and Holmes MC (2000). Inhibition of 11ß-hydroxysteroid dehydrogenase, the feto-placental barrier to maternal glucocorticoids, permanently programs amygdala glucocorticoid receptor mRNA expression and anxiety-like behavior in the offspring. *Europ J Neurosci* 12: 1047-1054.

Jamieson PM, Chaman KE, Walker BR and **Seckl JR** (2000). 11ß-hydroxysteroid dehydrogenase type 1 is a predominant 11ß-reductase in the intact perfused rat liver. *J Endocrinol* 165: 685-692.

Williams LJS, Lyons V, MAcLeod I, Rajan V, Darlington GJ, Poli V, **Seckl JR** and Chapman KE (2000). C/EBP☐ regulates hepatic transcription of 11ß-hydroxysteroid dehydrogenase type 1; a novel mechanisms for cross-talk between the C/EBP and glucocorticoid signalling pathways. *J Biol Chem* 275: 30232-30239.

Harris HJ, Kotelevtsev Y, Mullins JJ, **Seckl JR** and Holmes MC (2001). 11ß-hydroxysteroid dehydrogenase type 1 null mice have altered hypothalamic-pituitary-adrenal axis activity: a novel control of glucocorticoid feedback. *Endocrinology* 142: 114-120.

REVIEWS AND CHAPTERS

Seckl JR (1993). 11ß-HSD isoforms and their implications for blood pressure regulation. *Eur J Clin Invest* 23: 589-601.

Seckl JR and Brown RW (1994). 11ß-hydroxysteroid dehydrogenase: on several roads to hypertension. *J Hypertens* 12: 105-112.

Seckl JR and Olsson T (1995). Glucocorticoids and the age-impaired hippocampus: cause or effect? *J Endocrinol* 145: 201-211.

Yau JLW and **Seckl JR** (1995). Corticosteroids and the brain. *Curr Opin Endocrinol Diabetes*: 2: 239-247.

Edwards CRW, Benediktsson R, Lindsay RS and **Seckl JR** (1996). 11beta-hydroxysteroid dehydrogenases: Key enzymes in determining tissue-specific glucocorticoid effects. *Steroids* 61: 263-269.

Seckl JR (1997). 11ß-hydroxysteroid dehydrogenase: regulator of glucocorticoid action in the brain. *Front Neuroendocrinol* 18: 49-99

Chapman KE, Kotelevtsev YV, Jamieson PM, Williams LJS, Mullins JJ and **Seckl JR** (1997). Tissue-specific modulation of glucocorticoid action by the 11beta-hydroxysteroid dehydrogenases. *Biochem Soc Trans* 25: 583-587.

Seckl JR and Chapman KE (1997). Medical and physiological aspects of the 11ß-hydroxysteroid dehydrogenase system. *Eur J Biochem* 249: 361-364.

Seckl JR and Nyirenda MJ (1999). Glucocorticoids, feto-placental 11ß-hydroxysteroid dehydrogenase and the programming of hypertension. *Handbook of Hypertension* Vol. 19: Development of the Hypertensive Phenotype; McCarty R, Blizard DA, Chevalier RL (eds); Elsevier, Amsterdam, pp103-136.

Seckl JR (2000). 11ß-hydroysteroid dehydrogenases. *Encyclopaedia of Stress*. Fink G (ed). (in press).

Seckl JR and Walker BR (2001). 11ß-hydroxysteroid dehydrogenase type 1: a tissue-specific amplifier of glucocorticoid action. *Endocrinology* (in press).

Seckl JR and Walker BR (eds) (2001). Steroid Metabolism (book). *Bailliere's Clinical Endocrinology and Metabolism* (in press).

CURRICULUM VITAE **Dr Brian Robert Walker** **DOB 12/7/63 British**

Degrees etc

1984	BSc (1st class Hons)	Immunology	University of Edinburgh
1986	MB ChB		University of Edinburgh
1989	MRCP (UK)		
1993	MD (with Distinction)		University of Edinburgh
1999	FRCP Edinburgh		

Current Appointments

British Heart Foundation Senior Research Fellow (since Nov 1996)
Honorary Consultant Physician, Western General Hospital (since Nov 1996)
Member of Scientific Advisory Board, Wellcome Trust Cardiovascular Research Initiative and Centre for Cardiovascular Biology, University of Edinburgh (since Aug 1998)
Associate Director, Clinical Research Centre, University of Edinburgh (since June 1999)
Reader in Medicine, University of Edinburgh (since Oct 1999)
Director, GCMS Core Laboratory, Wellcome Trust Clinical Research Facility, Edinburgh (since Dec 1999)

Previous Appointments

1993-6	Lecturer in Medicine	University of Edinburgh
1992-3	Sir Stanley Davidson Lecturer in Medicine	University of Edinburgh
1989-93	MRC Training Fellow	University of Edinburgh
1987-89	SHO Rotation	Western Infirmary Glasgow

Postgraduate Prizes

William Leslie Prize for research awarded by University of Edinburgh Faculty of Medicine, 1991
Shortlisted for Young Investigator Award at the British Hypertension Society, Dublin, 1991
Finalist in Medical Research Society Young Investigator Prize competition, London, 1993
Wilfrid Card Lectureship and Medal, Edinburgh, 1994
Young Endocrinologist Award at the British Endocrine Societies, Warwick, 1995
Poster Prize at the Society for Endocrinology, London, 1995
Young Investigator Award at the International Society of Hypertension, Glasgow, 1996
Merck Senior Fellow Award at the International Congress of Endocrinology, San Francisco, 1996
British Hypertension Society Cardiovascular Research Travelling Fellowship to visit University of Umea, Sweden, 1997
Young Investigator Award at the British Hypertension Society, Bristol, 1997
Short-listed for the Austin Doyle Award at the International Society for Hypertension, Amsterdam, 1998
Special Travel Award to the International Society for Hypertension, Chicago, 2000

Academic recognition and activities

Member of Editorial Board for Clinical Endocrinology 1999-
Senior Editor for Journal of Endocrinology 2000-

Refereed grant applications for British Heart Foundation, Wellcome Trust, Medical Research Council, and British Diabetic Association; reviewed numerous manuscripts for diverse journals; examined 5 postgraduate theses

In year 2000, invited to lecture in Umea (Sweden), Gothenburg (Sweden), Phoenix (USA), Monte Carle (Monaco), and Nice (France); also lecturer at British Endocrine Societies Joint Meetings in 1998 and 1999, and at Association of Clinical Biochemists in Glasgow 1998.

RELEVANT PUBLICATIONS

Peer-Reviewed Publications

Dr Walker is author of more than 50 peer-reviewed articles since 1990. The following are especially relevant to 11β-hydroxysteroid dehydrogenases:

1. Walker BR, Yau JL, Brett LP, Seckl JR, Monder C, Williams BC, Edwards CRW (1991) 11ß-Hydroxysteroid dehydrogenase in vascular smooth muscle and heart: implications for cardiovascular responses to glucocorticoids. *Endocrinology*, 129: 3305-3312.
2. Walker BR, Edwards CRW (1991) 11ß-Hydroxysteroid dehydrogenase and enzyme-mediated receptor protection: Life after liquorice? *Clinical Endocrinology*, 35: 281-289.
3. Walker BR, Connacher AA, Webb DJ, Edwards CRW (1992) Glucocorticoids and blood pressure: a role for the cortisol/cortisone shuttle in the control of vascular tone in man. *Clinical Science*, 83: 171-178.
4. Walker BR, Moisan M-P (1992) Multiple isoforms of the cortisol-cortisone shuttle. *Journal of Endocrinology*, 133: 1-3.
5. Walker BR, Campbell JC, Williams BC, Edwards CRW (1992) Rapid Communication: Tissue-specific distribution of the NAD^+-dependent isoform of 11ß-hydroxysteroid dehydrogenase. *Endocrinology*, 131: 970-972.
6. Walker BR, Campbell JC, Fraser R, Stewart PM, Edwards CRW (1992) Mineralocorticoid excess and inhibition of 11ß-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. *Clinical Endocrinology*, 37: 483-492.
7. Walker BR, Stewart PM, Shackleton CHL, Padfield PL, Edwards CRW (1993) Deficient inactivation of cortisol by 11ß-hydroxysteroid dehydrogenase in essential hypertension. *Clinical Endocrinology*, 39: 221-227.
8. Walker BR, Sang KS, Williams BC, Edwards CRW (1994) Direct and indirect effects of carbenoxolone on responses to glucocorticoids and noradrenaline in rat aorta. *Journal of Hypertension*, 12: 33-39.
9. Walker BR, Williams BC, Edwards CRW (1994) Regulation of 11ß-hydroxysteroid dehydrogenase activity by the hypothalamic-pituitary-adrenal axis in the rat. *Journal of Endocrinology*, 141: 467-472.
10. Walker BR, Aggarwal I, Padfield PL, Stewart PM, Edwards CRW (1995) Endogenous inhibitors of 11ß-hydroxysteroid dehydrogenase in hypertension. *Journal of Clinical Endocrinology and Metabolism*, 80: 529-533.

11. Walker BR, Williamson PM, Brown MA, Honour JW, Edwards CRW, Whitworth JA (1995) 11ß-Hydroxysteroid dehydrogenase and its inhibitors in hypertensive pregnancy. *Hypertension*, 25: 626-630.
12. Walker BR, Connacher AA, Lindsay RM, Webb DJ, Edwards CRW (1995) Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. *Journal of Clinical Endocrinology and Metabolism*, 80: 3155-3159.
13. Stewart PM, Walker BR, Holder G, O'Halloran D, Shackleton CHL (1995) 11ß-Hydroxysteroid dehydrogenase activity in Cushing's syndrome: explaining the mineralocorticoid excess state of the ectopic adrenocorticotropin syndrome. *Journal of Clinical Endocrinology and Metabolism*, 80: 3617-3620.
14. Best R, Nelson SM, Walker BR (1997) Dexamethasone and 11-dehydrodexamethasone as tools to investigate the isozymes of 11ß-hydroxysteroid dehydrogenase *in vitro* and *in vivo*. *Journal of Endocrinology*, 153: 41-48.
15. Best R, Walker BR (1997) Additional value of measurement of urinary cortisone and unconjugated cortisol metabolites in assessing the activity of 11β-hydroxysteroid dehydrogenase *in vivo*. *Clinical Endocrinology*, 47: 231-236.
16. Walker BR, Best R, Noon JP, Watt GCM, Webb DJ (1997) Seasonal variation in glucocorticoid activity in healthy men. *Journal of Clinical Endocrinology and Metabolism*, 82: 4015-4019.
17. Walker BR, Phillips DIW, Noon JP, Panarelli M, Best R, Edwards HV, Holton DW, Seckl JR, Webb DJ, Watt GCM (1998) Rapid Communication: Increased glucocorticoid activity in men with cardiovascular risk factors. *Hypertension*, 31: 891-895.
18. Andrew R, Phillips DIW, Walker BR (1998) Rapid Communication: Obesity and gender influence cortisol secretion and metabolism in man. *Journal of Clinical Endocrinology and Metabolism*, 83: 1806-1809.
19. Walker BR, Andrew R, MacLeod KM, Padfield PL (1998) Growth hormone replacement inhibits renal and hepatic 11β-hydroxysteroid dehydrogenases in ACTH-deficient patients. *Clinical Endocrinology*, 49: 257-263.
20. Jamieson PM, Nyirenda MJ, Walker BR, Chapman KE, Seckl JR (1999) Interactions between oestradiol and glucocorticoid regulatory effects on liver-specific glucocorticoid-inducible genes: possible evidence for a role of hepatic 11ß-hydroxysteroid dehydrogenase type 1. *Journal of Endocrinology*, 160: 103-109.
21. Andrews RC, Walker BR (1999) Glucocorticoids and insulin resistance: old hormones, new targets. *Clinical Science*, 96: 513-523.
22. Jamieson A, Wallace AM, Walker BR, Andrew R, Fraser R, White PC, Connell JMC (1999) Apparent cortisone reductase deficiency: a functional defect in 11β-hydroxysteroid dehydrogenase type 1. *Journal of Clinical Endocrinology and Metabolism*, 84: 3570-3574.
23. Finken MJJ, Andrews RC, Andrew R, Walker BR (1999) Cortisol metabolism in healthy young adults: sexual dimorphism in activities of A-ring reductase but not 11β-hydroxysteroid dehydrogenases. *Journal of Clinical Endocrinology and Metabolism*, 84: 3316-3321.
24. Walker BR, Rodin A, Taylor NF, Clayton RN (2000) Endogenous inhibitors of 11β-hydroxysteroid dehydrogenase type 1 do not explain abnormal cortisol metabolism in polycystic ovarian syndrome. *Clinical Endocrinology*, 52: 77-80.
25. Livingstone DEW, Jones GC, Smith K, Jamieson PM, Andrew R, Kenyon CJ, Walker BR (2000) Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. *Endocrinology*, 141: 560-563.
26. Jamieson PM, Walker BR, Chapman KE, Rossiter S, Seckl JR (2000) 11β-Hydroxysteroid dehydrogenase type 1 is a predominant 11β-reductase in the intact perfused rat liver. *Journal of Endocrinology*, 165: 685-692.

27. Baker RW, Walker BR, Shaw RJ, Honour JW, Zumla A, Lightman SL, Rook GAW (2000) Increased cortisol/cortisone ratio in acute pulmonary tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 162: 1641-1647.
28. Mangos G, Walker BR, Kelly JJ, Lawson J, Webb DJ, Whitworth JA (2000) Cortisol inhibits cholinergic dilatation in the human forearm: towards an explanation for glucocorticoid-induced hypertension. *American Journal of Hypertension*, 13: 1155-1160.
29. Irving RJ, Belton NR, Elton RA, Walker BR (2000) Adult cardiovascular risk factors in premature babies. *Lancet*, 355: 2135-2136.
30. Ebrecht M, Buske-Kirschbaum A, Hellhammer DH, Kern S, Rohleder N, Walker BR, Kirschbaum C (2000) Tissue specificity of glucocorticoid sensitivity in healthy adults. *Journal of Clinical Endocrinology and Metabolism*, 85: 3733-3739.
31. Livingstone DEW, Kenyon CJ, Walker BR (2000) Mechanisms of dysregulation of 11β-hydroxysteroid dehydrogenase type 1 in Zucker obese rats. *Journal of Endocrinology*, 167: 533-539.
32. Yong PYK, Thong KJ, Andrew R, Walker BR, Hillier SG (2000) Development related increase in cortisol biosynthesis by human granulosa cells. *Journal of Clinical Endocrinology and Metabolism*, 85: 4728-4733.
33. Reynolds RM, Walker BR, Phillips DIW, Sydall HE, Andrew R, Wood PJ, Whorwood CB (2001) Altered control of cortisol secretion in adult men with low birthweight and cardiovascular risk factors. *Journal of Clinical Endocrinology and Metabolism*, 86: 245-250.
34. Rasmuson S, Andrew R, Nasman B, Seckl JR, Walker BR, Olsson T (2001) Increased cortisol production rate and altered glucocorticoid metabolism in women with mild to moderate Alzheimer's Disease. *Biological Psychiatry*, (in press).
35. Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DEW, Johnson O, Walker BR (2000) Tissue-specific dysregulation of cortisol metabolism in obesity. *Journal of Clinical Endocrinology and Metabolism*, (in press).
36. Seckl JR, Walker BR (2001) 11β-Hydroxysteroid dehydrogenase type 1: tissue-specific amplifier of glucocorticoid action. *Endocrinology*, (in press).
37. Reynolds RM, Walker BR, Sydall HE, Wood PJ, Phillips DIW (2001) Elevated plasma cortisol in glucose intolerant men: different responses to glucose and habituation to venepuncture. *Journal of Clinical Endocrinology and Metabolism*, (in press).

Non-Peer-Reviewed Articles and Book Chapters

Dr Walker is author of more than 30 reviews and book chapters relating to glucocorticoid biology, including the following which are most relevant to 11β-hydroxysteroid dehydrogenases:

1. Walker BR, Edwards CRW (1992) Clinical disorders of 11ß-hydroxysteroid dehydrogenase activity. In Recent Advances in Endocrinology and Metabolism (Volume 4) edited by Edwards CRW & Lincoln D. Churchill Livingstone, Edinburgh; pp 21-38.
2. Walker BR, Edwards CRW (1993) 11ß-Hydroxysteroid dehydrogenase activity in hypertension and renal disease. *Advances in Nephrology*, 22: 329-347.
3. Edwards CRW, Walker BR, Benediktsson R, Seckl JR (1993) Congenital and acquired syndromes of apparent mineralocorticoid excess. *Journal of Steroid Biochemistry and Molecular Biology*, 45: 1-5.
4. Walker BR (1993) Defective enzyme-mediated protection of corticosteroid receptors: novel mechanisms in the pathophysiology of hypertension. *Clinical Science*, 85: 257-263.
5. Stewart PM, Whorwood CB, Walker BR (1993) Steroid hormones and hypertension: the cortisol - cortisone shuttle. *Steroids*, 58: 614-620.

6. Edwards CRW, Walker BR (1993) Cortisol and hypertension: what was not so apparent about 'Apparent Mineralocorticoid Excess'. *Journal of Laboratory and Clinical Medicine*, 122: 632-635.
7. Walker BR, Edwards CRW (1994) Hypermineralocorticoidism due to abnormal metabolism of cortisol. *Current Opinions in Endocrinology and Diabetes*, 1: 109-116.
8. Walker BR (1994) Organ-specific actions of 11ß-hydroxysteroid dehydrogenase in humans: implications for the pathophysiology of hypertension. *Steroids*, 59: 84-89.
9. Walker BR, Edwards CRW (1994) Licorice-induced hypertension and syndromes of apparent mineralocorticoid excess. *Endocrinology and Metabolism Clinics of North America*, 23: 359-377.
10. Walker BR, Best R (1995) Clinical investigation of 11ß-hydroxysteroid dehydrogenase. *Endocrine Research*, 21: 379-387.
11. Benediktsson R, Walker BR, Edwards CRW (1995) Cellular selectivity of aldosterone action: role of 11ß-hydroxysteroid dehydrogenase. *Current Opinions in Nephrology and Hypertension*, 4:41-46.
12. Walker BR, Stewart PM, Edwards CRW (1997) Enzyme modulation of access to corticosteroid receptors. In Principles of Medical Biology edited by Bittar EE & Bittar N. JAI Press, Greenwich, Connecticut; Vol 10A, 261-274.
13. Walker BR (1996) Abnormal glucocorticoid activity in subjects with risk factors for cardiovascular disease. *Endocrine Research*, 22: 701-708.
14. Walker BR (1997) Modulation of glucocorticoid activity by metabolism of steroids in non-lymphoid organs. In: Steroid hormones and the T-cell cytokine profile. Rook GAW and Lightman S (eds) Springer-Verlag, London; pp 71-99.
15. Walker BR (1998) Glucocorticoid sensitivity in humans. *Current Opinion in Endocrinology and Diabetes*, 5: 168-174.
16. Walker BR (1999) Cortisol secretion and sensitivity in insulin resistance. In Insulin Resistance and Cardiovascular Disease, O'Rahilly S (ed), Bioscientifica, Bristol, 29-40.
17. Rook G, Baker R, Walker B, Honour J, Jessop D, Hernandez-Pando R, Arriaga K, Shaw R, Zumla A, Lightman S (1999) Local regulation of glucocorticoid activity in sites of inflammation; insights from the study of tuberculosis. *Annals of the New York Academy of Sciences*, (in press).
18. Walker BR (2000) Cortisol in insulin resistance syndromes. In The Diabetes Annual 13, Butler PC, Rizza RA, Walker M (eds), Elsevier Science, Amsterdam, (in press).
19. Walker BR (2000) How will we know if 11β-hydroxysteroid dehydrogenases are important in common diseases? *Clinical Endocrinology*, 52: 401-402..
20. Walker BR, Seckl JR (2000) Cortisol metabolism. In International Textbook of Obesity, Bjorntorp P (ed), John Wiley & Sons, Chichester, (in press).

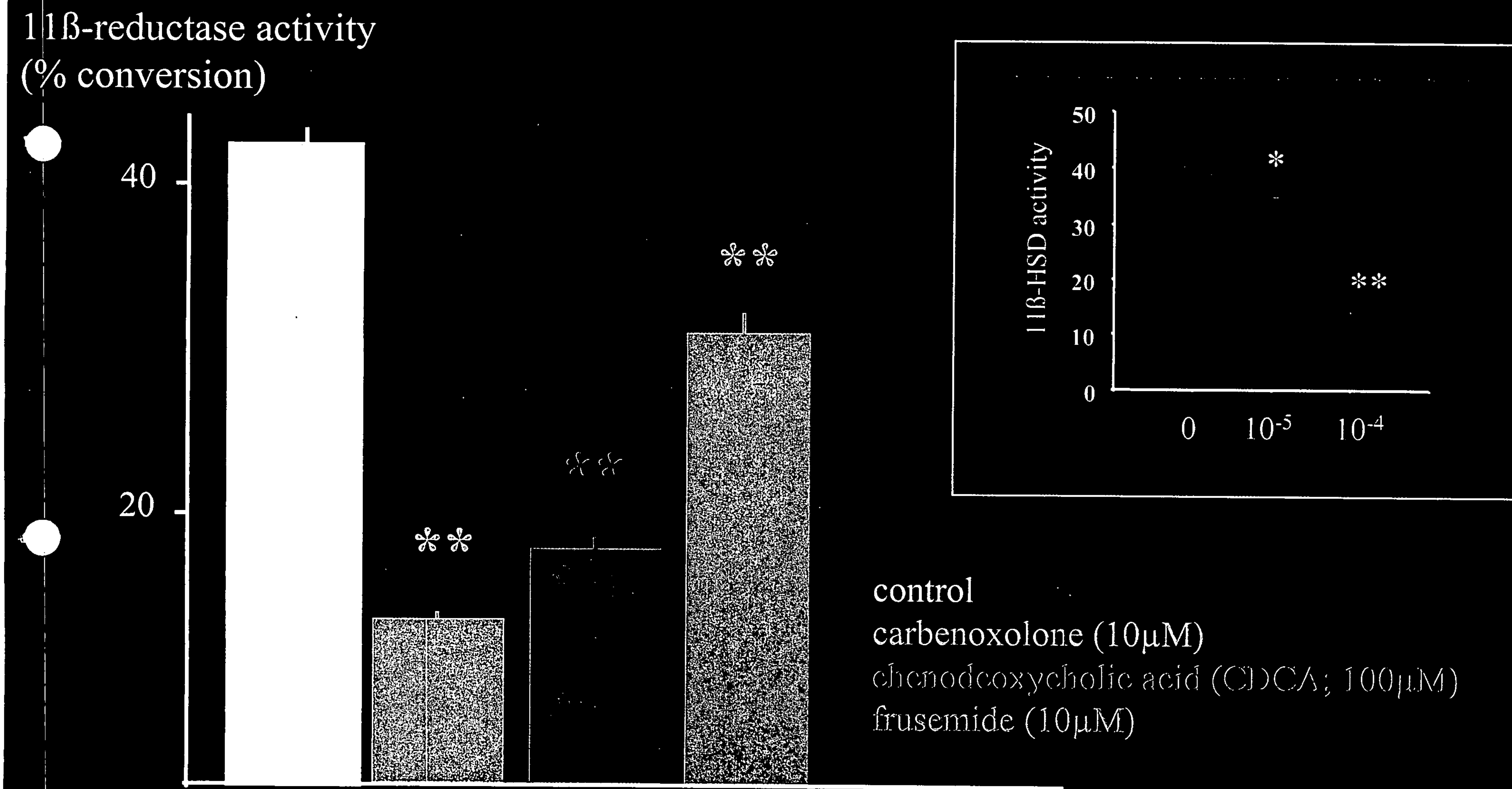
Books

1 Seckl JR, Walker BR (eds)(2000) Bailliere's Best Practice and Research in Clinical Endocrinology and Metabolism: Disorders of Steroid Metabolism. Bailliere Tindall, London, (in press).

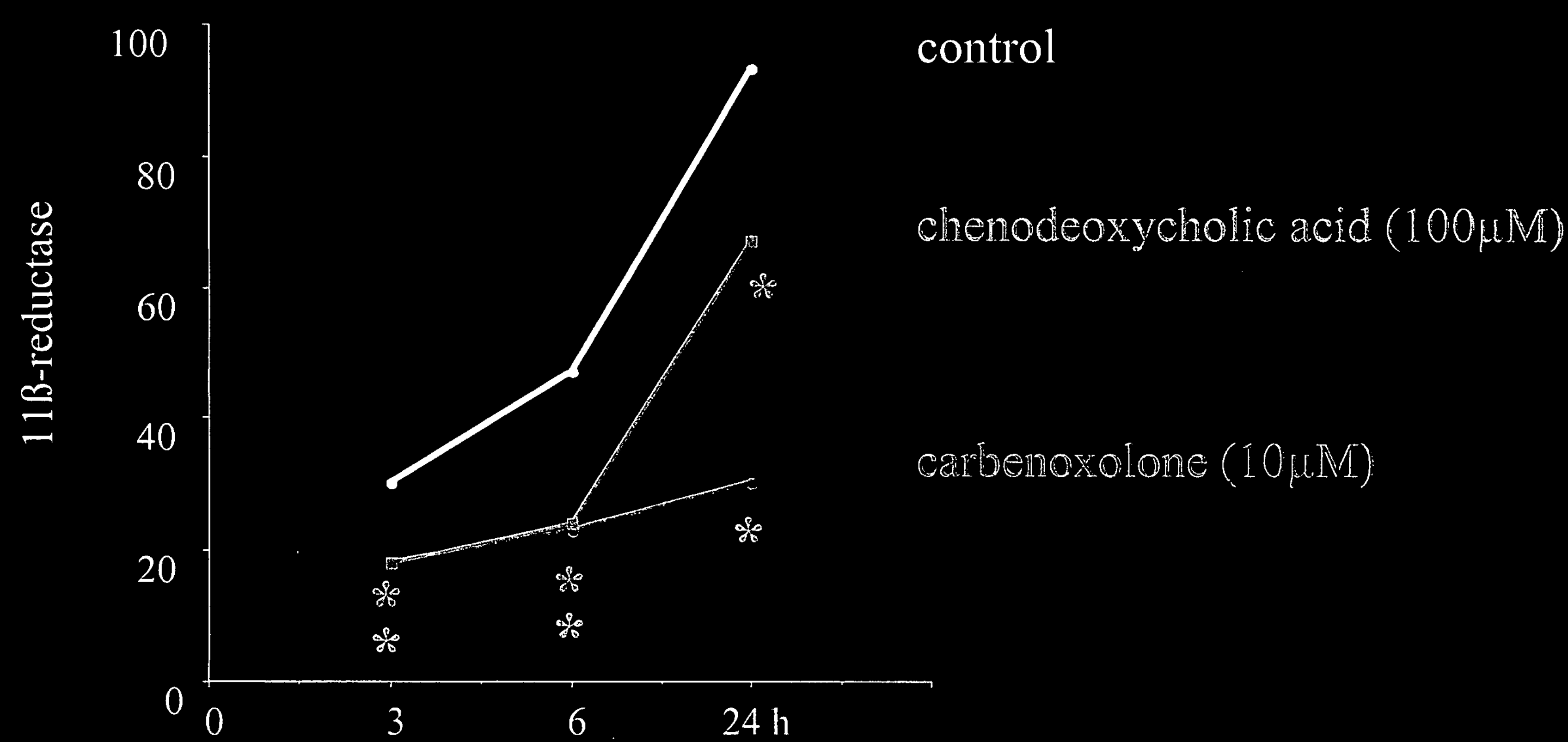
Abstracts & Letters

Dr Walker is author of >130 published abstracts and letters.

Some known inhibitors of 11ß-HSD also inhibit 11ß-reductase in intact primary neurons



Chenodeoxycholic acid inhibits 11ß-reductase in intact adipocytes



Following teachings in the application, we have identified further inhibitors of the reductase activity of 11ß-HSD1 in adipose and neuronal tissue.

ADVANCES IN PHYSIOLOGY EDUCATION
See Part 3, S1–S54

DECEMBER 1992
Volume 263/Number 6
Part 1 of Three Parts
ISSN 0002-9513

American Journal of Physiology

PUBLISHED BY
THE AMERICAN PHYSIOLOGICAL SOCIETY

Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone

ELISE P. GOMEZ-SANCHEZ AND CELSO E. GOMEZ-SANCHEZ
Research Service and Department of Internal Medicine, James A. Haley Veterans Hospital, and University of South Florida Health Science Center, Tampa, Florida 33612

Gomez-Sanchez, Elise P., and Celso E. Gomez-Sanchez. Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone. *Am. J. Physiol.* 263 (*Endocrinol. Metab.* 26): E1125–E1130, 1992.—The apparent mineralocorticoid excess syndrome of patients ingesting large amounts of licorice or its derivatives is thought to be caused by the antagonism by these compounds of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD inactivates cortisol and corticosterone, allowing the more abundantly produced glucocorticoids access to the mineralocorticoid receptor (MR) in the kidney, where they act as mineralocorticoids. We have found that the infusion of both glycyrrhizic acid, an active principle of licorice, and carbenoxolone, a synthetic analogue, into a lateral ventricle of the brain [intracerebroventricular (icv)] of a rat, at a dose less than that which has an effect when infused subcutaneously, produces hypertension. Furthermore, the hypertension produced by the oral administration of carbenoxolone or glycyrrhizic acid is blocked by the icv administration of RU 28318, an MR antagonist, at a dose below that which has an effect on blood pressure when infused subcutaneously. While the oral administration caused saline polydipsia and polyuria typical of chronic systemic mineralocorticoid excess, the icv licorice derivatives produced hypertension without affecting saline appetite. Sensitizing the rats to mineralocorticoid hypertension by renal mass reduction and increasing salt consumption was not necessary for the production of hypertension. These findings provide additional evidence for a central role in blood pressure control by mineralocorticoids that is distinct from their renal effects. They also suggest that more is involved in licorice-induced hypertension than only inhibition of 11β-HSD.

hypertension; licorice; mineralcorticoids; RU 28318; steroid 11β-hydroxysteroid dehydrogenase

ALDOSTERONE acts through type I receptors, or mineralocorticoid receptors (MR), in the kidney to produce sodium retention and potassium and hydrogen ion excretion. The MR is widely distributed and is present in the colon, parotid, vasculature, and, in particular, specific areas of the brain (5, 13). The affinity of isolated MR from various sources, including expressed MR cDNA in COS cells, is similar for aldosterone, corticosterone, and cortisol (3, 4, 16). MR, regardless of the source, are physicochemically identical (16, 32), and appear to be a product of the same cDNA (3). Corticosterone and cortisol normally do not act as mineralocorticoids in the kidney in vivo. Specificity, originally thought to be intrinsic to the receptor, has been shown to be conferred extrinsically by corticosterone/cortisol-binding globulin (CBG), which reduces free circulating glucocorticoid available to the receptor, and by 11β-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD reversibly converts corticosterone and cortisol to the inactive 11-dehydrocorticosterone and cortisone (7, 9, 12). The location of the 11β-HSD enzyme has been controversial. It appears that 11β-HSD is expressed in some mineralocorticoid target cells along with the MR, thus serving as an autocrine control, as well as in cells proximate to MR-containing cells, serving a paracrine function (6, 9, 21, 24).

Under normal conditions, most MR in the rat brain are almost fully occupied by corticosterone, while occupation of the type II receptor, or glucocorticoid receptor (GR), for which corticosterone has less affinity, is less complete and follows the circadian rhythm of glucocorticoid levels (7). It has been suggested that the occupation of the MR in the brain, particularly in the hippocampus, by corticosterone at low, physiological serum levels is possible because CBG does not penetrate the blood-brain barrier (7, 9) and because the activity of 11β-HSD in this organ is negligible (9, 12). However, in situ hybridization techniques have demonstrated the presence of 11β-HSD in the brain (19), as well as the kidney. Whether 11β-HSD is bioactive in any, all, or only specific parts of the brain is controversial (9, 19, 21). There are different tissue-specific forms and regional activity of the 11β-HSD enzyme (20) that may account for the apparent "glucocorticoid-selective" MR in some parts, particularly the hippocampus, of the brain, in contrast to the "aldosterone-preferring" MR in the anterior hypothalamus (7, 18). Seckl et al. (27) have reported that 11β-HSD inhibition by glycyrrhetinic acid in vivo in rats increased 2-deoxy-[^{14}C]glucose use in those areas of the brain where 11β-HSD mRNA expression has been documented. Corticosterone and aldosterone have different actions in some areas of the brain, even though both are thought to be acting with the same affinity and through the same receptor. Aldosterone antagonizes important central nervous system (CNS) effects of corticosterone (7, 26); corticosterone blocks the hypertension induced by the intracerebroventricular (icv) infusion of aldosterone (13, 15).

Apparent mineralocorticoid excess is a rare hypertensive syndrome in which patients have all of the manifestations of excessive production of mineralocorticoids, including hypokalemia, but steroid measurements are normal or low. The defect has been identified as a deficiency in 11β-HSD (11, 28, 30, 31). The pseudohyperaldosteronism, including hypokalemia and low-renin hypertension, produced by excessive licorice consumption and the treatment of peptic ulcers with licorice derivatives or their synthetic analogues has been attributed to the inhibition of this enzyme, allowing the more abundant circulating cortisol/corticosterone access to the MR in the kidney (9). Licorice derivatives and the synthetic analogue carbenoxolone have been used to study the mechanisms responsible for the syndrome of apparent mineralocorticoid excess, as well as the extrinsic factors conferring apparent ligand specificity to the MR (8, 10, 22). We herein describe studies of the central and

systemic effects of the icv, subcutaneous (sc), and oral administration of glycyrrhizic acid, a derivative of licorice, and carbenoxolone, a synthetic analogue, on the blood pressure using the specific MR antagonist RU 28318 (14) to inhibit the MR.

METHODS

Cannulas were placed into the right lateral cerebral ventricles of male outbred Sprague-Dawley rats weighing 180-200 g, using aseptic surgical technique under a combination of fentanyl and droperidol (Innovar-Vet, Pitman-Moore), 0.01 ml/100 g body wt sc, as preanesthetic and isoflurane as anesthetic. Rats received standard food (0.3% NaCl) and tap water or 0.9% saline ad libitum to amplify the hypertension as detailed below. Implanted miniosmotic pumps (Alzet 2002, Alza, Palo Alto, CA), which delivered 0.49 ± 0.02 μl/h for 14 days, were used for icv and sc infusions. Pumps were changed on *day 14* under isoflurane anesthesia, and pumps of the same lot were used throughout the experiment to ensure consistency. Carbenoxolone, RU 28318, and corticosterone were dissolved in cerebrospinal fluid (CSF) or 0.86% NaCl with 10% propyleneglycol for icv and sc infusion. A potassium gluconate solution that delivered the same amount of K^+ as the RU 28318 solution was used as control for the mineralocorticoid antagonist experiments (14). Reagents were purchased from Sigma, except for the RU 28318, which was a gift from Roussell (Romaineville, France). All solutions were made and sterilized by filtration through 0.2-μm filters (Acrodisc 13, Gelman Scientific) immediately before filling and implanting the pumps. Oral carbenoxolone or glycyrrhizic acid was administered individually twice a day as 0.1 or 0.2 ml of a slurry mixed in corn syrup that the rats accepted readily. Indirect systolic blood pressures (IITC, Woodhills, CA) and weights were measured twice a week starting before treatment as described previously (13). Twenty-four- or forty-eight-hour urine volumes were measured once a week in a stainless steel rat metabolism cage.

Effect of icv administration of carbenoxolone: dose response. Carbenoxolone was infused icv at a rate of 0.3, 1.0, and 3.0 μg/h and sc at a rate of 3.0 μg/h into intact rats provided with 0.9% saline to drink ad libitum.

Effect of icv administration of carbenoxolone and corticosterone. Carbenoxolone was infused icv at a rate of 5.0 μg/h and corticosterone at a rate of 20 ng/h, alone and together. Two types of experiments were done. For one, the rats were intact and drank tap water ad libitum. For the other, the right kidneys were removed and the rats drank 0.9% saline ad libitum to be comparable to the classical maneuvers used to amplify mineralocorticoid hypertension.

Effect of oral administration of carbenoxolone with and without icv RU 28318. Carbenoxolone was administered orally in corn syrup 45 mg/kg twice daily for 10 days and increased to 90 mg/kg twice daily for the next 4 days to ascertain that the hypertensive effect was maximal; the control rats received corn syrup orally. RU 28318 was infused icv at 1.1 μg/h in one-half of the animals receiving carbenoxolone; the other animals received a potassium gluconate solution to supply the equivalent amount of K^+ icv. We have previously shown that 1.1 μg/h RU 28318 icv has no intrinsic effect on the blood pressure but protects the rat from the hypertension of systemic mineralocorticoid excess, while being well below the dose required to affect on the blood pressure when infused sc (13, 14). The rats were intact and drank tap water ad libitum.

Effect of oral administration of glycyrrhizic acid with and without RU 28318. The effects of both glycyrrhizic acid and carbenoxlone were studied because of evidence that carbenoxolone may have a larger range of effects, including the inhibition of 11-oxoreductase, than does glycyrrhizic acid (29).

Glycyrrhizic acid was administered orally in corn syrup 35 mg/kg twice daily for 14 days. RU 28318 was infused icv and sc at 1.1 μg/h in two of three glycyrrhizic acid groups; the other glycyrrhizic acid animals received a potassium gluconate solution icv to supply the equivalent amount of K^+ icv. Another group received corn syrup orally and the potassium gluconate solution icv. The rats were intact and drank tap water ad libitum.

Animals were killed at the end of the studies by CO_2 narcosis and asphyxiation. Autopsies, including dye infusions to check cannula placement, were done at the conclusion of the study, and data from any animal in which there was doubt about the delivery of the solutions or which had evidence of illness causing undue stress were eliminated from the experiment. At the time of the biweekly pump changes, if the catheter was found to be disconnected from the pump or cannula, the data from the preceding two weeks were discarded and the animal eliminated from the study. We started with 8-10 animals per group so that the groups were never reduced to fewer than 7 animals by the end of the experiment. Data were compared by analysis of variance and the Dunnett *t* and Fisher PLSD tests (StatView 512+, BrainPower, Calabazas, CA).

RESULTS

Carbenoxolone, 3 μg/h administered icv to intact rats drinking 0.9% saline ad libitum, increased the blood pressure of rats significantly ($P < 0.01$) within 3 days and was maximal by *day 5* (Fig. 1). There was no significant change in the blood pressure of rats receiving 0.3 μg/h CSF, or 1 μg/h carbenoxolone icv or 3 μg/h carbenoxolone sc over 14 days. No significant difference was found in rate of weight gain or 24-h urine volume between any groups in the icv studies. In separate studies it was found that doses of carbenoxolone >5 μg/h resulted in precipitation of the drug in the pump and cannulas.

The icv infusion of corticosterone at 20 ng/h, a dose known to inhibit the hypertension produced by the icv infusion of aldosterone (15) while having no effect in and of itself, did not significantly blunt the increase in blood pressure produced by icv carbenoxolone, nor did it have any effect on the blood pressure by itself (Fig. 2). There was no difference in urine volume or weight gain between groups in the same experiments. Removing one kidney and giving saline to drink did not alter the hypertension

Fig. 1. Effect on indirect systolic blood pressure (BP) at *day 5* of intracerebroventricular (icv) and subcutaneous (sc) infusion of carbenoxolone (Cx) at 3.0 μg/h in intact rats drinking 0.9% saline ad libitum. CSF, cerebrospinal fluid.

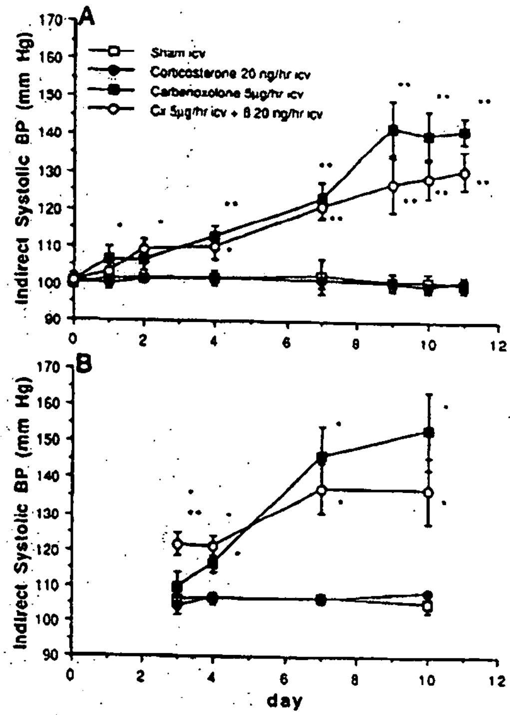


Fig. 2. Effect on indirect systolic blood pressure of icv infusion of carbenoxolone at 5.0 µg/h and corticosterone at 20 ng/h, alone and together, in nonsensitized rats (*A*; intact and drinking tap water ad libitum) compared with sensitized rats (*B*; one kidney removed and drinking 0.9% saline ad libitum). β, 11β-hydroxysteroid dehydrogenase. * $P < 0.05$. ** $P < 0.01$.

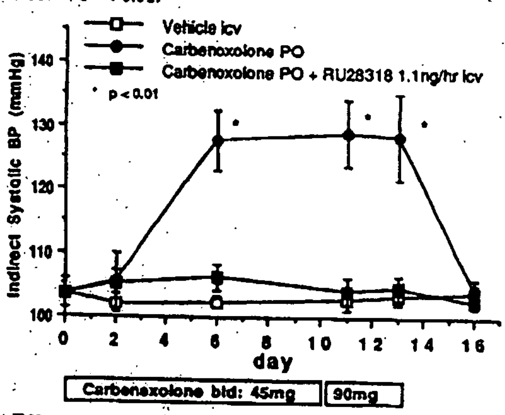


Fig. 3. Effect on indirect systolic blood pressure of oral administration of carbenoxolone in corn syrup at 45 mg/kg twice daily for 10 days, then 90 mg/kg twice daily for the next 4 days, while receiving an icv infusion of either RU 28318 at 1.1 ng/h or vehicle, in intact rats drinking tap water ad libitum.

produced by icv carbenoxolone or the effect of icv corticosterone. At *day 11* of the sensitization study there was a 41 and 39% difference in blood pressure between the controls and the icv carbenoxolone and icv carbenoxolone plus corticosterone, respectively, compared with 41 and 31% increases for the nonsensitized rats.

The blood pressure of intact rats drinking water and receiving oral carbenoxolone at 45 mg/kg twice daily increased significantly within 6 days from 105 mmHg to a plateau of 127 mmHg (Fig. 3). Doubling the dose to 90 mg/kg twice daily did not further increase the blood pressure. The icv infusion of 1.1 µg/h RU 28318 completely prevented the increase in blood pressure. We have shown in multiple studies, including those described below using glycyrrhizic acid instead of carbenoxolone, that the sc infusion of 1.1 µg/h RU 28318 is too low to affect the blood pressure. We have also reported that the icv infusion of the antagonist at three times this dose has no effect on the blood pressure of normal animals (14). The blood pressure in the animals receiving the icv control solution returned to normal within 3 days of discontinuing the oral administration of carbenoxolone. Orally administered carbenoxolone doubled the urine volume; this increase in urine volume was not prevented by the icv administration of the mineralocorticoid antagonist, which abolished the hypertension (Fig. 4). There was no difference in weight gain between groups.

The oral administration of glycyrrhizic acid at 35 mg/kg twice daily also significantly increased the blood pressure of intact rats drinking tap water. The icv infusion of 1.1 µg/h RU 28318 prevented the rise in blood pressure (Fig. 5). There was no difference in weight gain between groups.

DISCUSSION

The importance of the CNS in the development of mineralocorticoid hypertension has been well documented (5, 13). MR are found in the hippocampus, amygdala, lateral septum, and hypothalamus, particularly in the periventricular regions, areas known to be or suspected of being important in the regulation of adrenocorticotropic hormone (ACTH) release, arousal, fluid and fluid osmolality equilibrium, and the maintenance of normal blood pressure. The chronic icv infusion of aldosterone at a dose two orders of magnitude less than that necessary to produce hypertension when infused sc has been reported to produce hypertension in rats and dogs (21). The icv infusion of the mineralocorticoid antagonist RU 28318, at doses that have no effect on the blood pressure when given icv alone and that are ineffective as

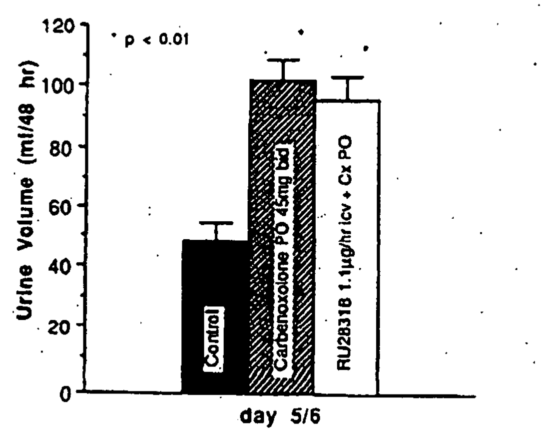


Fig. 4. Effect on 24-h urine volume of oral administration of carbenoxolone in corn syrup at 45 mg/kg twice daily for 10 days, then 90 mg/kg twice daily for the next 4 days, while receiving an icv infusion of either RU 28318 at 1.1 ng/h or vehicle, in intact rats drinking tap water ad libitum.

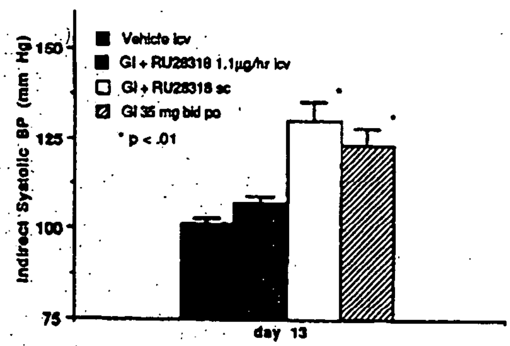


Fig. 5. Effect on indirect systolic blood pressure of oral administration of glycyrrhizic acid (GI) in corn syrup at 35 mg/kg twice daily, while receiving an icv infusion of either RU 28318 at 1.1 ng/h or vehicle, at *day 13* in intact rats drinking tap water ad libitum.

an antagonist when administered sc, blocks the hypertension of both the icv and systemic administration of aldosterone and the sc infusion of deoxycorticosterone acetate. The systemic, but not icv, aldosterone hypertension is associated with a chronically increased urine volume indicative of saline polydypsia/polyuria. The icv infusion of the antagonist prevents the rise in pressure produced by the systemic administration of aldosterone without preventing the associated polydypsia/polyuria (13, 14). These findings suggest distinct mineralocorticoid effects in the brain and kidney.

In the studies reported herein, the icv, but not sc, infusion of 3 μg/h carbenoxolone produced hypertension, implying that the site of action is in the brain. The hypertension produced by the oral and icv administration of carbenoxolone or glycyrrhizic acid resembles that of chronic systemic and icv aldosterone infusion in the amplitude of the increase in blood pressure and the effectiveness of mineralocorticoid receptor blockade by icv RU 28318 (13, 14). In addition, as with aldosterone, an increase in urine volume occurred only with the systemic, and not icv, administration of hypertensinogenic amounts of both licorice compounds. Blocking the hypertension of animals receiving oral carbenoxolone with the icv infusion of RU 28318 at doses too low to be effective when infused sc did not reduce their increase in urine volume. Classically mineralocorticoid-salt hypertension is associated with an initial retention of sodium and water followed by an "escape" from further retention and the establishment of a new equilibrium at a higher overall fluid volume. Polydypsia/polyuria may persist after reaching a balance with no additional net gain in water (13). Assuming that the carbenoxolone when given orally is causing a mineralocorticoid excess syndrome as far as the kidneys are concerned, one would expect initial sodium and water retention, followed by escape. The rats in these studies apparently were placed in metabolism cages after the water retention phase, assuming it occurred, after an equilibrium had been reached, because their urine output was consistently greater, not less, than that of controls. Weight gains were "real," not water gains, as evidenced by the fact that the weights of the oral carbenoxolone rats did not fall after the drug was withdrawn.

There was a consistent difference in the time of onset of the hypertension. Icv aldosterone hypertension takes from 7 to 11 days to become significant (13), while icv carbenoxolone hypertension was evident in 3–6 days. Considering the relatively long delay of onset, that of days rather than minutes or hours, it seems unlikely that this difference is due to a more rapid passage of the licorice compounds across the blood-brain barrier; it probably reflects a more basic difference in the mechanism of action. Removing one kidney and giving saline to drink did not exacerbate the hypertension produced by icv carbenoxolone. This was surprising because the classical way to amplify mineralocorticoid hypertension is to reduce renal mass and increase sodium consumption and because in the model of central mineralocorticoid hypertension, equihypertensinogenic doses of icv aldosterone in nonsensitized rats were nine times that of sensitized rats (13).

Glycyrrhizic acid and carbenoxolone are not thought to act as agonists at the receptor level because their affinity for the MR is negligible (2). They are presumed to work by inhibiting 11β-HSD, thereby removing the protection of the MR from corticosterone and allowing it to act as a mineralocorticoid (12). However, if 11β-HSD were active in the brain, and if it were inhibited by carbenoxolone, previous studies from our laboratory suggest that the resulting accumulation of corticosterone would not be expected to increase blood pressure. An additional difference between the icv aldosterone and icv carbenoxolone models is that the icv infusion of corticosterone, at a dose that would have been expected from our previous work to antagonize the icv aldosterone model, had no effect on the blood pressure of rats receiving icv carbenoxolone. It is assumed that the inhibitory action of icv corticosterone on icv aldosterone hypertension is mediated by the MR because RU 26988, a selective GR agonist, had no effect when infused alone or in combination with aldosterone (15).

While most reported studies indicate that carbenoxolone does not affect the mineralocorticoid activity of aldosterone (25), others suggest that it enhances the sodium retention produced by aldosterone and 11-deoxycorticosterone (23). Glycyrrhetinic acid has been found to inhibit the hepatic 5β-reductase and 3β-HSD but not the 5α-reductase or 3α-HSD. Another proposed mechanism for the enhancement of mineralocorticoid activity by licorice derivatives is the accumulation of aldosterone, deoxycorticosterone, and 11-deoxycorticosterone and their biologically active 5α-dehydro derivatives due to the inhibition of the 5β-reductase and 3β-HSD enzymes, as well as of glucocorticoids due to 11β-HSD inhibition (17).

Patients with apparent mineralocorticoid excess appear to be deficient in 11β-dehydrogenase but not 11-oxoreductase enzyme activity (30). While it has been assumed that 11β-HSD is an enzyme complex consisting of an 11β-dehydrogenase and a distinct 11-oxoreductase (9, 22, 30), a rat cDNA has been cloned and expressed as a single enzyme that interconverts cortisol/corticosterone to cortisone/11-dehydrocorticosterone (1). It has been reported that glycyrrhizic acid and carbenoxolone are not identical in their clinical activities and that glycyrrhizic acid inhibits the conversion of cortisol/corticosterone

to cortisone/11-dehydrocorticosterone unidirectionally, while carbenoxolone inhibits both the dehydrogenase and reductase directions (29). In our studies, the activity of glycyrrhizic acid and carbenoxolone were similar.

There is evidence for yet another mechanism of action of carbenoxolone. The MR is either missing or defective in patients with pseudohypoaldosteronism. Funder (10) has reported that the administration of carbenoxolone with a selective GR agonist in patients with pseudohypoaldosteronism and in adrenalectomized rats alters the function of the glucocorticoid, causing it to produce the same renal effects, Na^+ retention and K^+ excretion, as a mineralocorticoid would, presumably by causing GR-ligand complexes to act as activated MR. The animals in our experiments had intact adrenals; in fact, the mineralocorticoid effects of licorice depend on intact adrenal glands or replacement corticosteroids (9). Normally, most of the MR and many of the GR of the brain, depending on the area, are tonically bound by corticosterone, even in the unstressed rat (7, 26). While the concomitant icv infusion of corticosterone blocks icv aldosterone hypertesion, the icv infusion of a selective glucocorticoid, presumably to the GR only, does not antagonize icv aldosterone hypertension. If there are two classes of MR in the brain, as has been postulated by De Kloet (7), carbenoxolone and glycyrrhizic acid may be altering the "corticosterone-preferring" MR to functionally "aldosterone-preferring" MR. If carbenoxolone were producing hypertension by "recruiting" GR and/or corticosterone-preferring MR bound to endogenous corticosterone to the pool of functionally activated MR, not only might the same cellular response be elicited as by activated MR in a mineralocorticoid-sensitive central blood pressure control area, but, more important, it might also remove the receptors that mediate the inhibition of icv aldosterone hypertension. This might explain why icv corticosterone, when given with carbenoxolone, neither increased the blood pressure, because the receptors were already surfeited, nor decreased it, because they were being diverted from their usual role of buffering the hypertensinogenic effect of aldosterone. The more rapid induction of hypertension by licorice compounds compared with aldosterone may be due more to the removal of local inhibitory effects than to the recruitment of more functional MR. The yin-yang relationship of the two classes of corticosteroids has been described elsewhere, including in the brain (7).

These data provide additional evidence for a central role in blood pressure control by mineralocorticoids that is distinct from their renal effects and that involves a complex homeostatic relationship between the two classes of corticosteroids in their central effects on blood pressure. They suggest that our understanding of functional specificity of the corticosteroid receptor-ligand complex, particularly in the brain, is incomplete. Finally, these studies indicate that more is involved in licorice-induced hypertension than the inhibition of 11β-HSD.

The expert technical help of Chris Fort is gratefully acknowledged.
These studies were supported by Medical Research Funds from the Department of Veterans Affairs and a by National Heart, Lung, and Blood Institute Grant HL-27737.

Address for reprint requests: E. P. Gomez-Sanchez, Research Service (151R), James A. Haley Veterans Hospital, 13000 Bruce B. Downs Blvd., Tampa, FL 33612.

Received 24 February 1992; accepted in final form 24 July 1992.

REFERENCES

1. **Agarwal, A. K., C. Monder, B. Eckstein, and P. C. White.** Cloning and expression of rat cDNA encoding corticosteroid 11 β-dehydrogenase. *J. Biol. Chem.* 264: 18939–18943, 1989.
2. **Armanini, D., I. Karbowiak, and J. W. Funder.** Affinity of liquorice derivatives for mineralocorticoid and glucocorticoid receptors. *Clin. Endocrinol.* 19: 609–612, 1983.
3. **Arriza, J. L., R. B. Simerly, L. W. Swanson, and R. M. Evans.** The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. *Neuron* 1: 887–900, 1988.
4. **Beaumont, K., and D. D. Fanestil.** Characterization of rat brain aldosterone receptors reveals high affinity for corticosterone. *Endocrinology* 113: 2043–2051, 1983.
5. **Bohr, D. F.** What makes the pressure go up? A hypothesis. *Hypertension Dallas* 3, *Suppl.* II: II-160–II-165, 1981.
6. **Castello, R., R. Schwarting, C. Muller, K. Hierholzer, and I. Lichtenstein.** Immunohistochemical localization of 11-hydroxysteroid dehydrogenase in rat kidney with a monoclonal antibody. *Renal Physiol. Biochem.* 12: 320–327, 1989.
7. **De Kloet, R. E.** Brain corticosteroid receptor balance and homeostatic control. *Front. Neuroendocrinol.* 12: 95–164, 1991.
8. **Doyle, D., R. Smith, S. Krozowski, and J. W. Funder.** Mineralocorticoid specificity of renal type I receptors: binding and metabolism of corticosterone. *J. Steroid Biochem.* 33: 165–170, 1989.
9. **Edwards, C. R. W., and P. M. Stewart.** The cortisol-cortisone shuttle and the apparent specificity of glucocorticoid and mineralocorticoid receptors. *J. Steroid Biochem. Mol. Biol.* 39: 859–865, 1991.
10. **Funder, J. W.** How can aldosterone act as a mineralocorticoid? *Endocrinol. Res.* 15: 227–238, 1989.
11. **Funder, J. W., P. T. Pearce, K. Myles, and L. P. Roy.** Apparent mineralocorticoid excess, pseudohypoaldosteronism, and urinary electrolyte excretion: toward a redefinition of mineralocorticoid action. *FASEB J.* 4: 3234–3238, 1990.
12. **Funder, J. W., P. T. Pearce, R. Smith, and A. I. Smith.** Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science Wash. DC* 242: 583–585, 1988.
13. **Gomez-Sanchez, E. P.** What is the role of the central nervous system in mineralocorticoid hypertension? *Am. J. Hypertens.* 4: 374–381, 1991.
14. **Gomez-Sanchez, E. P., C. M. Fort, and C. E. Gomez-Sanchez.** Intracerebroventricular infusions of RU 28318 blocks aldosterone-salt hypertension. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E482–E484, 1990.
15. **Gomez-Sanchez, E. P., M. T. Venkataraman, and D. Thwaites.** Icv infusion of corticosterone antagonizes icv-aldosterone hypertension. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E649–E653, 1990.
16. **Krozowski, Z. S., and J. W. Funder.** Renal mineralocorticoid receptors and hippocampal corticosterone binding species have identical intrinsic steroid specificity. *Proc. Natl. Acad. Sci. USA* 80: 6056–6060, 1983.
17. **Latif, S. A., T. J. Conca, and D. J. Morris.** The effects of the licorice derivative, glycyrrhetinic acid, on hepatic 3α- and 3β-hydroxysteroid dehydrogenases and 5α- and 5β-reductase pathways of metabolism of aldosterone in male rats. *Steroids* 55: 52–58, 1990.
18. **McEwen, B. S., L. T. Lambdin, T. C. Rainbow, and A. F. De Nicola.** Aldosterone effects on salt appetite in adrenalectomized rats. *Neuroendocrinology* 43: 38–43, 1986.
19. **Moisan, M. P, J. R. Seckl, and C. R. W. Edwards.** 11β-Hydroxysteroid dehydrogenase bioactivity and messenger RNA expression in rat forebrain: localization in hypothalamus, hippocampus, and cortex. *Endocrinology* 127: 1450–1455, 1990.
20. **Monder, C., and V. Lakshmi.** Evidence for kinetically distinct forms of corticosteroid 11β-dehydrogenase in rat liver microsomes. *J. Steroid Biochem.* 32: 77–83, 1989.
21. **Monder, C., and V. Lakshmi.** Corticosteroid 11β-dehydrogenase of rat tissues: immunological studies. *Endocrinology* 126:

2435–2443, 1990.

22. **Monder, C., P. M. Stewart, V. Lakshmi, R. Valentino, D. Burt, and C. R. W. Edwards.** Licorice inhibits corticosteroid 11β-dehydrogenase of rat kidney and liver: in vivo and in vitro studies. *Endocrinology* 89: 1046–1252, 1989.
23. **Morris, D. J., and G. W. Souness.** The 11β-OHSD inhibitor, carbenoxolone, enhances Na retention by aldosterone and 11-deoxycorticosterone. *Am. J. Physiol.* 258 (*Renal Fluid Electrolyte Physiol.* 27): F756–F759, 1990.
24. **Naray-Fejes-Toth, A., C. O. Watlington, and G. Fejes-Toth.** 11β-Hydroxysteroid dehydrogenase activity in the renal target cells of aldosterone. *Endocrinology* 129: 17–21, 1991.
25. **Porter, G. A., C. Rhodes, and P. Sacra.** Comparative studies on the mineralo-corticoid action of aldosterone and carbenoxolone sodium in the adrenalectomized rat. *Pharmacology* 12: 224–229, 1974.
26. **Reul, J. M. H. M., F. R. Van Den Bosch, and E. R. De Kloet.** Relative occupation of type-I and type-II corticosteroid receptors in rat brain following stress and dexamethasone treatment: functional implications. *J. Endocrinol.* 115: 459–467, 1987.
27. **Seckl, J. R., P. A. T. Kelly, and J. Sharkey.** Glycyrrhetinic acid, an inhibitor of 11β-hydroxysteroid dehydrogenase, alters local cerebral glucose utilization in vivo. *J. Steroid Biochem. Mol. Biol.* 39: 777–779, 1991.
28. **Stewart, P. M., J. E. T. Corrie, C. H. L. Shackleton, and C. R. W. Edwards.** Syndome of apparent mineralocorticoid excess. *J. Clin. Invest.* 82: 340–349, 1988.
29. **Stewart, P. M., A. M. Wallace, S. M. Atherdon, C. H. Shearing, and C. R. Edwards.** Mineralocorticoid activity of carbonoxolone: contrasting effects of carbenoxolone and liquorice on 11β-hydroxysteroid dehydrogenase activity in man. *Clin. Sci. Lond.* 78: 49–54, 1990.
30. **Ulick, S., L. S. Levine, and P. Gunczler.** A syndrome of apparent mineralocorticoid excess associated with defects in the peripheral metabolism of cortisol. *J. Clin. Endocrinol. Metab.* 49: 757–64, 1979.
31. **Ulick, S., R. Tedde, and F. Mantero.** Pathogenesis of the type 2 variant of the syndrome of apparent mineralocorticoid excess. *J. Clin. Endocrinol. Metab.* 70: 200–206, 1990.
32. **Wrange, O., and Z.-Y. Yu.** Mineralocorticoid receptor in rat kidney and hippocampus: characterization and quantitation by isoelectric focusing. *Endocrinology* 113: 243–250, 1983.

RAHAM
ıtagonists

KICHI,

in rats

D,

ilament
ıf the

ıvenging
cid in an

/ and

action
ts and

gonist
ve
n rats

ı and
ats

t

:als.
ant

ıd
ıd

Journal of PHARMACY & PHARMACOLOGY

Volume 46 · Number 4 · April 1994
ISSN 0022-3573

HABENDA RATIO VALETUDINIS

J. Pharm. Pharmacol. 1994, 46: 305-309
Received August 27, 1993
Accepted November 15, 1993

A Novel 11β-Hydroxysteroid Dehydrogenase Inhibitor Contained in Saiboku-To, a Herbal Remedy for Steroid-dependent Bronchial Asthma

MASATO HOMMA, KITARO OKA, TOMOYUKI NIITSUMA* AND HISAO ITOH*

*Department of Clinical Pharmacology, Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo 192-03, Japan, and *Third Internal Medicine, Tokyo Medical College Hospital, Shinjuku-ku, Tokyo 160, Japan*

Abstract—To identify the inhibitor of prednisolone metabolism contained in Saiboku-To, we conducted in-vitro experiments of 11β-hydroxysteroid dehydrogenase (11β-HSD), using rat liver homogenate and cortisol as a typical substrate. We studied the effects of ten herbal constituents on 11β-HSD. Five herbal extracts showed inhibitory activity with *Glycyrrhiza glabra* > *Perillae frutescens* > *Zizyphus vulgaris* > *Magnolia officinalis* > *Scutellaria baicalensis*. This suggests that unknown 11β-HSD inhibitors are contained in four herbs other than *G. glabra* which contains a known inhibitor, glycyrrhizin (and glycyrrhetinic acid). Seven chemical constituents which have been identified as the major urinary products of Saiboku-To in healthy and asthmatic subjects were studied; magnolol derived from *M. officinalis* showed the most potent inhibition of the enzyme (IC50, 1.8×10^{-4} M). Although this activity was less than that of glycyrrhizin, the inhibition mechanism (non-competitive) was different from a known competitive mechanism. These results suggest that magnolol might contribute to the inhibitory effects of Saiboku-To on prednisolone metabolism through inhibition of 11β-HSD.

Saiboku-To is the most popular anti-asthmatic Chinese herbal medicine (Kampo medicine in Japan) and has been used for corticosteroid-dependent asthma to obtain a steroid-sparing effect in prednisolone therapy (Nagano et al 1988). On the basis of animal experiments, the mechanism of action of Saiboku-To has been attributed to hormonal stimulation of the adrenal cortex (Hiai et al 1981; Shimizu et al 1984) and synergistic adjuvant effects on autacoid secretions (Toda et al 1988) or allergic reactions (type I and IV) (Nishiyori et al 1983, 1985).

Recently, we proposed another mechanism which involves suppression of the systemic elimination of prednisolone (Taniguchi et al 1992). This pharmacokinetic effect seemed to result from 11β-hydroxysteroid dehydrogenase (11β-HSD) metabolic enzyme inhibition, because plasma prednisolone/prednisone ratios following Saiboku-To administration increased significantly (Taniguchi et al 1992). Since other Kampo-preparations containing *Glycyrrhiza glabra* did not show an effect on prednisolone pharmacokinetics (unpublished data), the effect of Saiboku-To could not be explained by known enzyme inhibitors such as glycyrrhizin and its aglycone glycyrrhetinic acid, which are contained in *G. glabra*. These observations suggested that Saiboku-To must contain as yet uncharacterized 11β-HSD inhibitors.

In the present study, we carried out in-vitro experiments of 11β-HSD inhibition using cortisol and rat liver homogenate.

Materials and Methods

Materials

Saiboku-To (TJ-96, Tsumura Co., Tokyo, Japan) consists of fine brownish granules containing ten different herbal extracts (Table 1). Original herbs used for the assay were purchased from Uchida Wakanyaku Co. (Tokyo, Japan). The extracts of Saiboku-To and of original herbs were prepared as follows. One gram Saiboku-To or the crushed herb in 15 mL 35% ethanol was gently refluxed for 1 h on a steam bath. After cooling to room temperature, water was added to make a total volume of 10 mL before centrifugation at 1600 *g* for 10 min. The resulting supernatant was used for the assay.

Glycyrrhizin, glycyrrhetinic acid, wogonin, and baicalein were purchased from Wako Pure Chemicals (Osaka, Japan). Magnolol and honokiol were donated by Professor Y. Sashida of Tokyo College of Pharmacy (Fujita et al 1973). Medicarpin and oroxylin A were kindly contributed by Professor T. Nomura of Toho University School of Pharmacy (Tokyo, Japan) and Tsumura Co., respectively. 8,9-Dihydroxydihydromagnolol was prepared by us from magnolol by osmic acid oxidation (Homma et al 1992). Liquiritigenin was isolated from *G. glabra* according to Shibata & Saitoh (1978). Chemical structures of these compounds are given in Fig. 1. Cortisol and cortisone were purchased from Sigma Chemical Co. (St Louis, MO, USA). Other organic and inorganic reagents were of analytical grade.

Rat liver homogenates were prepared in the usual manner: fresh liver was isolated from a male Wistar rat (freely fed, body weight 250 g) and was cut into small pieces. The pieces were homogenized in 10 vol 0·25 M sucrose in a glass homogenizer with a Teflon piston. The homogenates were frozen at −80°C and stored until incubation.

Instruments

Our HPLC system for determination of glucocorticoids in incubation mixtures consisted of a solvent delivery pump (VIP-I, Jasco, Tokyo), a UV-detector (Uvidec-100-III, Jasco), a single pen recorder (Pantos U-228, Nippon Denshi, Tokyo), a sample injector with a loop volume of 100 μL

Correspondence: M. Homma, Department of Clinical Pharmacology, Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo 192-03, Japan.

Table 1. Herbal composition of Saiboku-To.

Constituent herb	Family	Composition (%, w/w)
Bupleurum falcatum L.	Umbelliferae	20·6
Pinellia ternata Beitenbach	Araceae	14·7
Poria cocos Wolf.	Polyporaceae	14·7
Scutellaria baicalensis Georgi	Labiatae	8·8
Zizyphus vulgaris Lam.	Rhamnaceae	8·8
Panax ginseng C. A. Meyer	Araliaceae	8·8
Magnolia officinalis	Magnoliaceae	8·8
Glycyrrhiza glabra L.	Leguminosae	5·9
Perillae frutescens Britton var. *acuta* Kudo	Labiatae	5·9
Zingiber officinale Roscoe	Zingiberaceae	3·0

(Model 7125, Rheodyne, CA, USA), and a silica gel column (LiChrosorb Si-60, 5 μm, i.d. 4 mm × 250 mm, Merck, Darmstadt, Germany). The mobile phase was a mixture of water/methanol/dichloromethane/n-hexane (0·1/8·0/30·0/61·9 v/v) with a flow rate of 1·5 mL min^{-1}. Detector sensitivity was set at 0·005–0·01 aufs at 245 nm. We used a disposable syringe minicolumn (Extrashot, Kusano Sci. Co., Tokyo) to perform sample injections (Homma et al 1989; Kouno et al 1990).

Determination of 11β-HSD inhibition activity

We measured 11β-HSD activity in rat liver homogenate incubation mixtures, detecting chemical transformation of cortisol to cortisone in the presence of 11β-HSD inhibitors. Oxidation at the C-11 position of the steroid nucleus was kinetically characterized by measuring the conversion rate of cortisol to cortisone in the presence of $NADP^+$ in rat liver homogenate according to the procedure of Monder et al (1989) with minor modification. The incubation mixtures consisted of 620 μL 0·1 M Tris-HCl buffer (pH 8·5) containing 0·014% Triton-X, 50 μL 5 mM $NADP^+$, 100 μL rat liver homogenate, and 200 μL aqueous solution for Saiboku-To and original herbal extracts or 200 μL buffer solution for each chemical such as the known inhibitors (glycyrrhizin and glycyrrhetinic acid) and our candidates isolated from urine of subjects receiving the preparation. These chemicals were dissolved in a buffer solution directly or after pre-solubilization in a small amount of ethanol with a final concentration in incubation mixtures of less than 2%. After 10 min pre-incubation at 37°C, 200 μL 0·3 mM cortisol was added and the resulting mixtures were further incubated for 10 min. The enzyme reaction was terminated by an addition of 100 μL 5% sulphuric acid. Cortisol and cortisone in the mixtures were determined by HPLC using Extrashot as described in our previous papers (Homma et al 1989; Kouno et al 1990). Briefly, 5 μL incubation mixture and 2 μL sodium hydroxide solution were loaded onto Extrashot which was then attached to the sample-loading injector of the HPLC system.

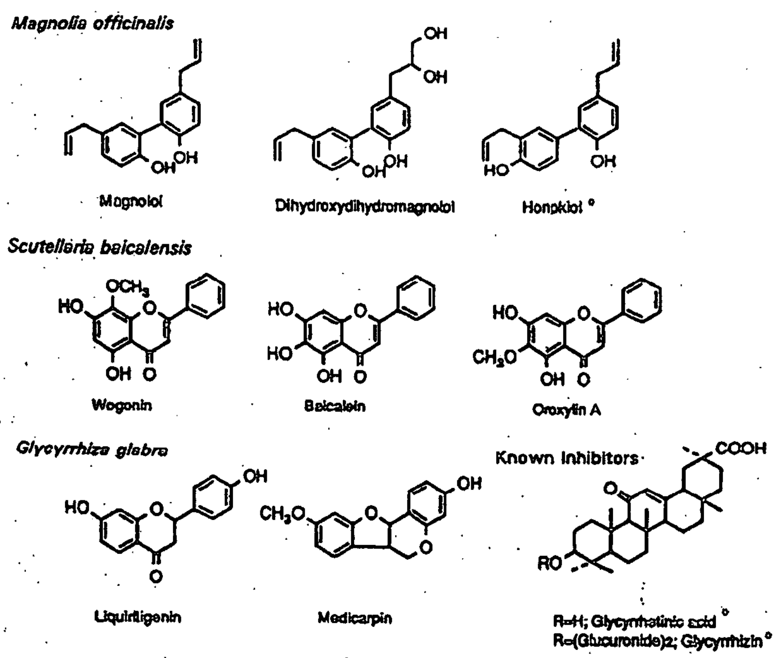


FIG. 1. Chemical structures of test compounds. * These compounds have not been detected in urine following Saiboku-To administration.

Ethanol/dichloromethane (2/98 v/v, 130 μL) was injected into the system through Extrashot using a tuberculin glass syringe. Thus, extraction and injection of the glucocorticoids in the incubation mixtures were achieved simultaneously. The recovery rates of glucocorticoids from the incubation mixture were more than 95% with coefficient of variations less than 5%. Direct peak-height calibration of the test and control mixtures afforded inhibitory activity (% inhibition) of the test materials against 11β-HSD.

Results

Effects of herbal extracts

Effects of original herbal extracts on conversion of cortisol to cortisone by rat liver homogenate were compared with that of Saiboku-To (Table 2). Cortisone production in the reaction mixture was significantly inhibited by Saiboku-To and five original herbal extracts ($P < 0.05$). The magnitude of the inhibition (% inhibition) was in the order Saiboku-To (87·5%) > *G. glabra* (80·8%) > *P. frutescens* (30·9%) > *Z. vulgaris* (27·6%) > *M. officinalis* (19·8%) > *S. baicalensis* (19·1%).

Effects of urinary metabolites of Saiboku-To

Seven candidates (Fig. 1) were tested with respect to the effects on rat liver 11β-HSD at concentrations of 10 and 100 μM. The results were compared with those of the known inhibitors, glycyrrhizin and glycyrrhetinic acid (Table 3). Five of seven candidates showed inhibitory activity at 100 μM, although their activities were weaker than those of the known inhibitors. Dihydroxydihydromagnolol in *M. officinalis* and liquiritigenin in *G. glabra* did not show any activity at the test concentrations. Wogonin, baicalein, and oroxylin A (flavonoids derived from *S. baicalensis*), and medicarpin (a

Table 2. Effects of Saiboku-To and its constituent herbal extracts on 11β-hydroxysteroid dehydrogenase in rat liver homogenate.

	% inhibition[a]	% activity of Saiboku-To
Saiboku-To	87·5±3·4**	100·0
B. falcatum	7·7±5·7	8·8
P. ternata	5·8±4·2	6·6
P. cocos	—	—
S. baicalensis	19·1±11·5*	21·8
Z. vulgaris	27·6±4·0**	31·5
P. ginseng	10·9±6·9	12·5
M. officinalis	19·8±3·7**	22·6
G. glabra	80·8±1·0**	92·3
P. frutescens	30·9±9·6**	35·3
Z. officinale	12·8±8·7	14·6

[a] Data are presented as mean ± s.d. of triplicate experiments. *$P < 0.05$, **$P < 0.01$ compared with control.

Table 3. Inhibition of 11β-hydroxysteroid dehydrogenase by urinary metabolites of Saiboku-To and known inhibitors.

	Inhibition (%)	
Inhibitor	10 μM	100 μM
Urinary metabolites of Saiboku-To		
Magnolol	15·1±4·4	43·9±3·0
Dihydroxydihydromagnolol	—	—
Wogonin	—	7·4±0·8
Baicalein	6·8±1·6	14·8±1·6
Oroxylin A	—	5·1±5·5
Liquiritigenin	—	—
Medicarpin	—	12·2±3·3
Known inhibitors		
Glycyrrhizin	81·1±5·4	97·3±1·1
Glycyrrhetinic acid	100·0	

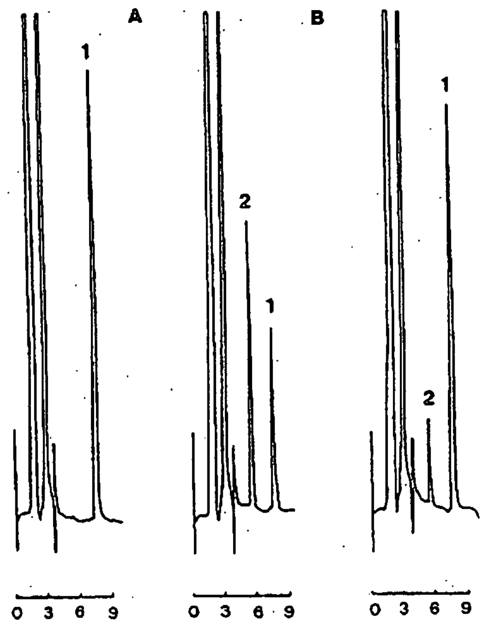


FIG. 2. Chromatographic comparison of the effect of magnolol (100 μM) on transformation of cortisol (peak 1) to cortisone (peak 2) by 11β-hydroxysteroid dehydrogenase. A. Before incubation with magnolol; B. after incubation without magnolol; C. after incubation with magnolol.

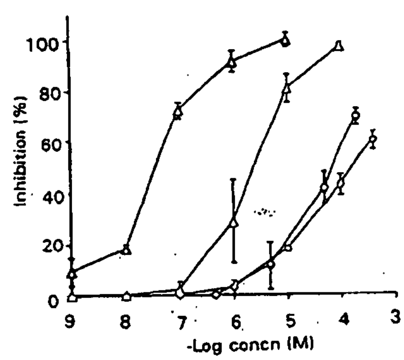


FIG. 3. Dose-dependent inhibitory effects of magnolol (○), honokiol (○), glycyrrhizin (△), and glycyrrhetinic acid (△) on 11β-hydroxysteroid dehydrogenase. Data are presented as mean ± s.d. of triplicate experiments.

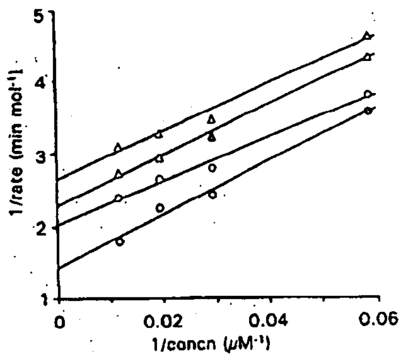


FIG. 4. Lineweaver–Burk double reciprocal plots of initial enzyme velocity and concentration of cortisol in the presence of magnolol at concentrations of 0 (O), 0·1 (O), 0·15 (Δ), and 0·2 (Δ) mM.

homoisoflavonoid in *G. glabra*) showed weak activity. However, considerable inhibition was observed with magnolol, a neolignan derived from *M. officinalis*. A typical chromatogram for determination of the inhibitory activity of magnolol is shown in Fig. 2, where the chemical transformation from cortisol to cortisone was clearly suppressed. The dose-dependent inhibitory effect of magnolol is compared with those of glycyrrhizin and glycyrrhetinic acid in Fig. 3. The IC50 values of magnolol, glycyrrhizin, and glycyrrhetinic acid were $1{\cdot}8\times10^{-4}$, $2{\cdot}6\times10^{-6}$, and $9{\cdot}0\times10^{-8}$ M, respectively. Since *M. officinalis* contains another congener of magnolol, honokiol (not a urinary metabolite), we also examined the effect of honokiol on 11β-HSD and found a dose-dependent inhibitory effect with IC50 of $7{\cdot}0\times10^{-5}$ M (Fig. 3).

Mechanism of magnolol in 11β-HSD inhibition

Fig. 4 shows the inhibitory effects of magnolol on rat liver 11β-HSD. The data were plotted according to the Lineweaver–Burk linear transformation of the Michaelis–Menten equation. The double reciprocal plots on Fig. 4 suggested magnolol has a unique non-competitive inhibitory mechanism. We were unable to estimate an inhibition constant (K_i) of magnolol by the Dixon plot because of this non-competitive inhibition.

Discussion

This paper suggests the presence of several novel inhibitors of 11β-HSD in five constituent herbs. *G. glabra, P. frutescens, Z. vulgaris, M. officinalis* and *S. baicalensis*. Although these inhibitors seem to contribute to in-vitro activity of Saiboku-To, their contributions to prednisolone metabolism during clinical Saiboku-To treatment has been unclear. However, we emphasize the importance of this possibility, since our biologically active compounds in herbal medicine are found in biofluids following administration (Homma et al 1992, 1993a).

In our previous study, we found seven phenolic compounds in urine after oral administration of Saiboku-To (Homma et al 1992, 1993a, b). These compounds seemed to be possible candidates which explain in-vivo effects of Saiboku-To. Five of these compounds showed inhibitory activity against 11β-HSD in-vitro (Table 3). The intensities of those activities were almost equal to those of the corresponding herbal extracts, except that *G. glabra*, containing glycyrrhizin, concealed the effects of liquiritigenin and medicarpin. Magnolol exhibited activity at concentrations higher than 1×10^{-5} M (Fig. 3). Similar activity was also observed in honokiol, a hydroxylated derivative of magnolol isolated from *M. officinalis* but not found as a urinary metabolite of Saiboku-To.

The novel 11β-HSD inhibitors found in this study belong to a class of phenolic compounds, lignans and flavonoids, whose chemical structures are completely different from those of the previously described inhibitors. Unexpectedly, the inhibition mechanism of magnolol seems to be different from those of the known inhibitors, the latter exhibiting competitive inhibition (Monder et al 1989). Although 11β-HSD inhibitors have been considered so far to belong to a limited class of liquorice triterpenoids, the present results suggested that the naturally occurring lignans and flavonoids also possess inhibitory activity through a different mechanism.

Urinary non-conjugated magnolol in responders to Saiboku-To is significantly higher than that in the non-responders (Homma et al 1993a, b). This suggests that magnolol is an important chemical constituent for the clinical effects of Saiboku-To, playing an important role for alteration of prednisolone pharmacokinetics.

The inhibitory effects of liquorice glycosides on 11β-HSD are so marked in animal experiments in-vivo and in-vitro (Monder et al 1989; Mackenzie et al 1990), that Saiboku-To could inhibit 11β-HSD even though the glycyrrhizin content is relatively small. However, the effect of Saiboku-To cannot be explained by glycyrrhizin alone, because another Kampo preparation, Sho-Saiko-To which contains *G. glabra* but not *P. cocos, M. officinalis* or *P. frutescens*, did not affect prednisolone pharmacokinetics in healthy subjects (unpublished data). Animal experiments using pure compounds will be needed to clarify the role of lignans and flavonoids on prednisolone metabolism.

Acknowledgements

This work was supported by the Ministry of Education in Japan (Grant-in-Aid for Scientific Research 03857345). Dr Y. Sashida and T. Nomura are gratefully acknowledged for kindly providing magnolol and honokiol and medicarpin. We thank Miss E. Yoshida and Mr H. Tamura for their technical assistance.

References

Fujita, M., Itokawa, H., Sashida, Y. (1973) Study on the components of *Magnolia obonata* thumb. II. On the components of the methanol extract of the bark. Yakugaku Zasshi 93: 422–428 (in Japanese)

Hiai, S., Yokoyama, H., Nagasawa, T., Oura, H. (1981) Stimulation of the pituitary-adrenocortical axis by saikosaponin of *Bupreuri radix*. Chem. Pharm. Bull. (Tokyo) 29: 495–499

Homma, M., Oka, K., Takahashi, N. (1989) Liquid chromatographic determination of theophylline concentration with syringe-type minicolumns for direct plasma injection. Anal. Chem. 61: 784–787

Homma, M., Oka, K., Yamada, T., Niitsuma, T., Itoh, H., Takahashi, N. (1992) A strategy for discovering biologically active compounds with high probability in traditional Chinese

herb remedies: an application of Saiboku-To in bronchial asthma. Anal. Biochem. 202: 179–187

Homma, M., Oka, K., Niitsuma, T., Itoh, H. (1993a) Pharmacokinetic evaluation of traditional Chinese herb remedies. Lancet 341: 1595

Homma, M., Oka, K., Kobayashi, H., Niitsuma, T., Yamamoto, S., Itoh, H., Takahashi, N. (1993b) Impact of free magnolol excretions in asthmatic patients who responded well to Saiboku-To, a Chinese herbal medicine. J. Pharm. Pharmacol. 45: 844–846

Kouno, Y., Ishikura, C., Takahashi, N., Homma, M., Oka, K. (1990) Direct sample injection into the high-performance liquid chromatographic column in theophylline monitoring. J. Chromatogr. 515: 321–327

Mackenzie, M. A., Hoetnagels, W. H. L., Jansen, R. W. M. M., Benrad, T. J., Kloppenborg, P. W. C. (1990) The influence of glycyrrhetinic acid on plasma cortisol and cortisone in healthy young volunteers. J. Clin. Endocrinol. Metab. 70: 1637–1643

Monder, C., Stewart, P. M., Lakshmi, V., Valentino, R., Burt, D., Edwards, C. R. W. (1989) Licorice inhibits corticosteroid 11β-dehydrogenase of rat kidney and liver: in vivo and in vitro studies. Endocrinology 125: 1046–1053

Nagano, J., Kobayashi, S., Nakajima, S., Egashira, Y. (1988) A study of the long-term effects of Saiboku-To in bronchial asthma—evaluation in the field of pediatrics by multi-center open trial. Respiration 7: 76–87 (in Japanese)

Nishiyori, T., Nakatomi, I., Matsuura, N., Nagai, H., Koda, A. (1983) Effect of Chinese blended medicine, Saiboku-To, on Type IV allergic reaction. Jpn. J. Allergol. 32: 317–323

Nishiyori, T., Tsuchiya, H., Inagaki, N., Koda, A. (1985) Effects of Chinese blended preparation, Saiboku-To, in type I allergic reaction and experimental atopic asthma in particular. Folia Pharmacol. Japon. 85: 7–16 (in Japanese)

Shibata, S., Saitoh, T. (1978) Flavonoid compounds in licorice root. J. Ind. Chem. Soc. 55: 1184–1191

Shimizu, K., Amagaya, S., Ogihara, Y. (1984) Combination effects of Sho-Saiko-To (Chinese traditional medicine) and prednisolone on the anti-inflammatory action. J. Pharmacobiodyn. 7: 891–899

Taniguchi, C., Homma, M., Oka, K., Kobayashi, H., Takahashi, N., Yamamoto, S., Itoh, H. (1992) Effects of Saiboku-To on prednisolone metabolism. Jpn. J. Ther. Drug Monit. 9: 18–24 (in Japanese)

Toda, S., Kimura, M., Ohnishi, M., Nakanishi, K. (1988) Effects of the Chinese herbal medicine "Saiboku-To" on histamine release from and the degranulation of mouse peritoneal mast cells induced by compound 48/80. J. Ethnopharmacol. 24: 303–309

JANUARY 1996

CLINICAL PHARMACOLOGY & THERAPEUTICS

OFFICIAL PUBLICATION OF THE
American Society for Clinical Pharmacology and Therapeutics
American Society for Pharmacology and Experimental Therapeutics

VOLUME 59 NUMBER 1

MARCUS M. REIDENBERG, MD *Editor*

APPEARING IN THIS ISSUE

Mosby
ISSN 0009-9236

Grapefruit juice and its flavonoids inhibit 11β-hydroxysteroid dehydrogenase

Introduction: The enzyme 11β-hydroxysteroid dehydrogenase (11β-OHSD) oxidizes cortisol to inactive cortisone. Its congenital absence or inhibition by licorice increases cortisol levels at the mineralocorticoid receptor, causing mineralocorticoid effects. We tested the hypothesis that flavonoids found in grapefruit juice inhibit this enzyme in vitro and that grapefruit juice itself inhibits it in vivo.
Methods: Microsomes from guinea pig kidney cortex were incubated with cortisol and nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) and different flavonoids and the oxidation to cortisone measured with use of HPLC analysis. In addition, healthy human volunteers drank grapefruit juice, and the ratio of cortisone to cortisol in their urine was measured by HPLC and used as an index of endogenous enzyme activity.
Results: Both forms of 11β-OHSD requiring either NAD or NADP were inhibited in a concentration-dependent manner by the flavonoids in grapefruit juice. Normal men who drank grapefruit juice had a fall in their urinary cortisone/cortisol ratio, suggesting in vivo inhibition of the enzyme.
Conclusion: Dietary flavonoids can inhibit this enzyme and, at high doses, may cause an apparent mineralocorticoid effect. (Clin Pharmacol Ther 1996;59:62-71.)

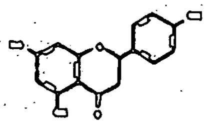
Yil Seob Lee, MD,[a] Beverly J. Lorenzo, BS, Theo Koufis, MS, and Marcus M. Reidenberg, MD *New York, N.Y.*

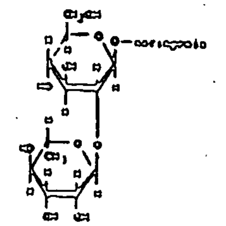
The enzyme 11β-hydroxysteroid dehydrogenase (11β-OHSD) oxidizes cortisol to inactive cortisone. This enzyme in the kidney regulates the amount of mineralocorticoid activity there, because cortisol binds as avidly to the mineralocorticoid receptor as aldosterone does. Deficiency of this enzyme in children, first described by Ulick et al.[1] in 1977, causes high cortisol levels in the kidney that result in hypertension and hypokalemia. Licorice-induced hypermineralocorticoidism is probably due to the inhibition of 11β-OHSD by glycyrrhizic acid, the active principle of licorice.[2-4] Much research has been done since 1977 on syndromes of apparent mineralocorticoid excess.[5,6]

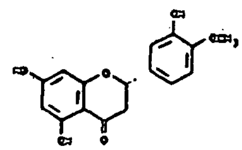
Gossypol, a polyphenolic constituent of cotton seed, has been studied in China as a potential male oral contraceptive, but hypokalemia developed in some Chinese men while they were taking it.[7] We found that gossypol inhibited 11β-OHSD activity in guinea pig[8] and human renal cortical microsomes.[9] Because there are structural similarities between gossypol and some flavonoids, we tested some of these and some other compounds, such as diuretics, that cause hypokalemia[9-10] and discovered that some inhibit this enzyme. Narin-

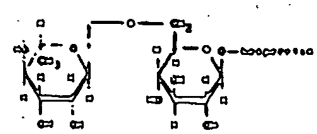
From the Departments of Pharmacology and Medicine, Division of Clinical Pharmacology, Cornell University Medical College.
Supported by grant RR47 from the National Institutes of Health (Bethesda, Md.) and by grants from Hoffmann-La Roche Inc. (Nutley, N.J.), Sandoz Pharmaceuticals Inc. (East Hanover, N.J.), The Rockefeller Foundation (New York, N.Y.), and Han-Dok Remedia (Seoul, Korea).
Received for publication May 11, 1995; accepted Aug. 17, 1995.
Reprint requests: Marcus M. Reidenberg, MD, Department of Pharmacology, Cornell University Medical College, 1300 York Ave., New York, NY 10021.
[a]Present address: Han-Dok Remedia Ind. Co., Ltd., 735 Yoksam-1-Dong, Kangnan-Ku, SL Young Dong, PO Box 1560, Seoul, Korea.

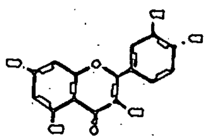
0009-9236/96/$5.00 + 0 13/1/68639

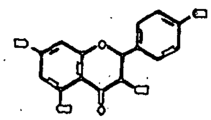

Naringenin

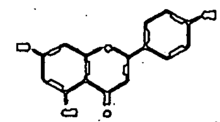

Naringin


Hesperetin


Hesperidin


Quercetin


Kaempferol


Apigenin

Structures of flavonoids.

genin, the aglycone of naringin, is a major flavonoid in grapefruit juice and inhibits this enzyme.[10] Recent work suggests that there are two isoforms of this enzyme, nicotinamide adenine dinucleotide (NAD)–dependent 11β-OHSD and nicotinamide adenine dinucleotide phosphate (NADP)–dependent 11β-OHSD with specific tissue distributions.[11-14] The effects of these flavonoids are worth study because about 25 mg of flavonoids has been recently estimated to be ingested daily in the diet,[15] whereas older studies cite as much as 1 gm per day.[16]

The objective of this study was to learn which other flavonoids in grapefruit juice inhibit 11β-OHSD in vitro and whether grapefruit juice inhibits the enzyme in vivo.

MATERIAL AND METHODS

In vitro study

Chemicals and solutions. All flavonoids (see Structures), cortisone, cortisol, corticosterone, NAD, NADP, 99.9% dimethyl sulfoxide (DMSO), and Sigma Diagnostic Total Protein Kit (cat. No. 690-A) were purchased from Sigma Chemical Co., St. Louis, Mo. All flavonoids were dissolved in DMSO. Cortisone, cortisol, and corticosterone were dissolved in methanol (J.T. Baker HPLC grade purchased from VWR Scientific, Piscataway, N.J.) (1.4 mmol/L) and kept at −4° C. NAD and NADP (5 mmol/L) were dissolved in Tris hydrochloric acid buffer (0.1 mol/L, pH 8.0).

Microsomal preparation and assay of 11β-OHSD activity. Guinea pig kidney cortex was obtained from long-haired male Hartley guinea pigs (Hilltop, Pa.). Tissue was homogenized by a Tekmar Tissuemizer (Cincinnati, Ohio). Microsomes were prepared, diluted to a concentration of 1.25 mg protein/ml as measured by the Sigma Diagnostics Total Protein Kit, and stored at −70° C. The enzyme activity in the microsomes was determined by measuring the rate of conversion of cortisol to cortisone in the presence of NAD or NADP as described previously.[8-10] Each flavonoid was studied with use of NAD and NADP as the cofactor. The conversion rates from cortisol to cortisone were determined, and the extent of inhibition was calculated. The drug concentrations that inhibited the enzyme by 50% (IC_{50}) were estimated from duplicate incubations at each concentration of at least three different concentrations of each flavonoid by use of the dose-response program of Chou and Chou (*Dose-effect Analysis with Microcomputers*, Elsevier-Biosoft, Cambridge, En-

gland, 1989). For each flavonoid studied, at least one concentration was above and one below the IC_{50}.

Analytical method for urinary cortisone and cortisol

We modified our HPLC method for measurement of these compounds from microsomal incubation mixtures.[9] The equipment consisted of a Waters Automated Gradient Controller with two Waters 6000A pumps (Waters Chromatography, Milford, Mass.). The injector was a Waters U6K and the detector was a Waters 486 Tunable Absorbance Detector set at a wavelength of 246 nm and 0.15 absorbance units full scale. The separation was performed with a Waters Nova-Pak C_{18} 3.9 × 150 mm stainless steel column (4 μm spherical particle size, pore size 60 Å, 7% carbon load, end-capped) or with a Waters μBondapak C_{18} 3.9 × 300 mm column (10 μm irregular particle size, pore size 125 Å, 10% carbon lead, end-capped). The peak areas were recorded on a SE120 plotter purchased through Waters Chromatography.

The mobile phase was methanol/water, initially at 70% water:30% methanol. Conditions were changed over the first 6 minutes to 56% water:44% methanol in a linear gradient that was then held isocratically for 14 minutes. The gradient was then reversed linearly to 70:30 over 3 minutes and the column equilibrated for 5 minutes before the next injection. The flow rate was 1 ml/min.

Procedure

To each 10 ml aliquot of every standard and sample (performed in duplicate) was added 40 μl of the 25 μg/ml corticosterone* as the internal standard. The samples were briefly vortexed to mix. One milliliter of 0.1 mol/L of sodium hydroxide was added to each test tube and again briefly vortexed to mix. Three milliliters of methylene chloride were added to each sample, capped with Teflon-lined screw tops, and rotated for 45 minutes on a mechanical rotator at approximately 20 rpm. The samples were centrifuged at 3000 rpm (1000*g*) for 15 minutes. The aqueous layer (top) was aspirated to waste. Again the samples were centrifuged for 10 minutes at 3000 rpm and the remainder of the aqueous phase was aspirated. A small spatula full of sodium chloride (~150 mg) was added to each sample, and any emulsion was broken up with a Pasteur pipet. The samples were then again centrifuged for 10 minutes. The organic layer was carefully transferred to clean test tubes and evaporated to dryness in a warm water bath (~45° C) under a stream of nitrogen. The residue was redissolved in 150 μl of HPLC grade methanol and injected into the HPLC.

The retention times were 16.5, 19.0, and 23.5 minutes for cortisone, cortisol, and corticosterone, respectively, on a Waters 10 micron, 300 × 3.9 mm stainless steel μBondapak C_{18} column. On a Waters 4 micron, 150 × 3.9 mm Nova-Pak, the retention times for cortisone, cortisol, and corticosterone were 12.8, 13.6, and 17.8 minutes. Levels measured in about 60 human urine samples ranged from 7.1 to 215.4 ng/ml for cortisone and 4.5 to 230.1 ng/ml for cortisol. The ratio of cortisone to cortisol was 0.2 to 5.7.

The absolute recovery was 70% for cortisol and 69% for cortisone. The interday coefficient of variation for cortisone was 6.5% for 25 ng/ml and 1.5% for the 100 ng/ml standard. For cortisol, the values were 6.3% for 25 ng/ml and 1.1% for 100 ng/ml. Cortisone dissolved in methanol was chromatographed and the peak was collected. The putative cortisone peak from extracted urine was also collected, and both fractions were scanned with a Varian Cary 219 spectrophotometer. The peaks had identical absorption spectra, with absorption maximums at 239 nm. (The *CRC Handbook of Chemistry and Physics* states that the absorption maximum of cortisone in alcohol is 237 nm).

All samples were assayed twice in duplicate. Standard curves for cortisone and cortisol were determined and plotted as in the in vitro study. Concentrations of these steroids in unknown samples were extrapolated from these standard curves.[10]

In vivo preliminary study

Six male volunteers aged from 35 to 65 years (two investigators and four other members of the Department of Pharmacology) who were living at home gave daily morning urine samples for 4 days. They then drank grapefruit juice, requested to be at a dose of a quart a day, for 7 days, and gave daily morning urine samples on the last 4 days of this period. After a 3-day washout period, the subjects again gave daily morning urine samples for 4 days.

*Corticosterone is excreted by humans at a rate that averages 6 μg/24 hours[17] or less than of 1% of 1.5 to 4.0 mg/24 hour production rate.[18] Thus the concentration from endogenous sources is less than 10% of that added, a negligible amount for this assay.

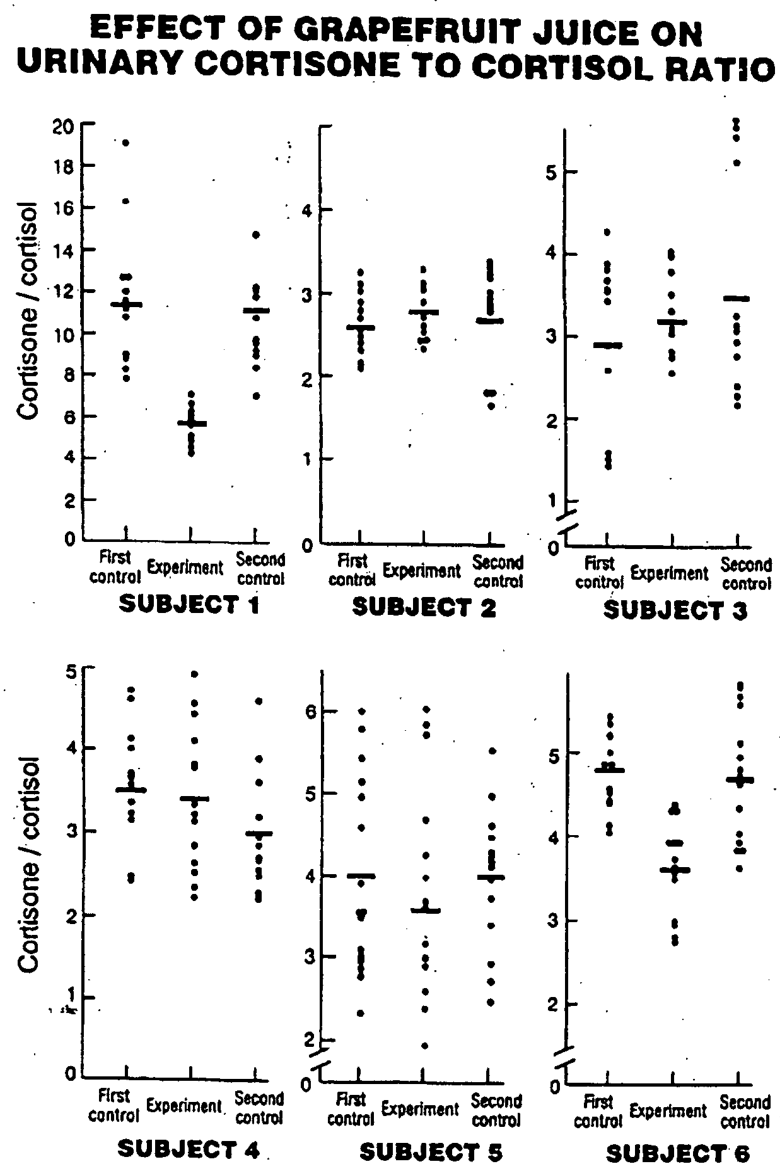


Fig. 1. Preliminary study results in six subjects living at home. Subjects 1 and 6 were two of the authors, who are known to have consumed the full amount of grapefruit juice.

The cortisone and cortisol concentrations were measured in each urine sample. The two investigators (subjects 1 and 6) had a decrease in the ratio of urinary cortisone to cortisol during the grapefruit juice period compared with the control periods before and after grapefruit juice (mean ± SD for subject 1 was 11.4 ± 3.1, 5.7 ± 0.9, and 10.2 ± 2.1; mean ± SD for subject 6 was 4.8 ± 0.4, 3.6 ± 0.6, and 4.7 ± 0.8). The other four subjects had no significant change. All data are shown in Fig. 1. Subjects 1 and 6 then volunteered for the dose-response study.

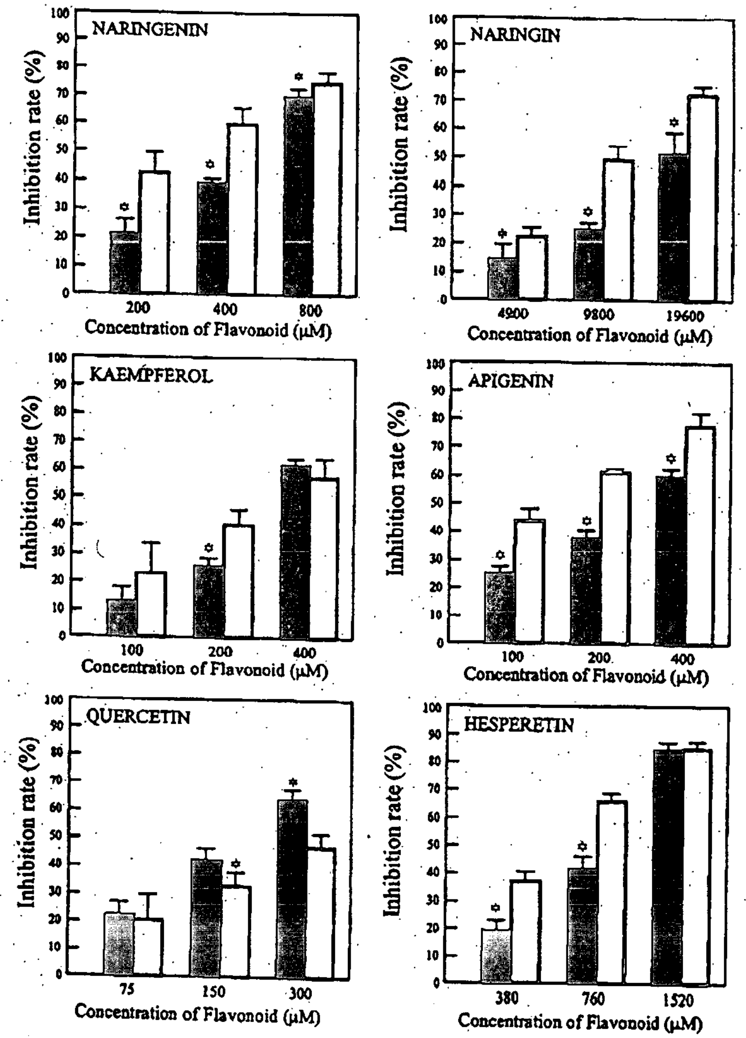


Fig. 2. Concentration-response relationships for the inhibition of 11β-hydroxysteroid dehydrogenase by different flavonoids with use of nicotinamide adenine dinucleotide (NAD; *solid bars*) or nicotinamide adenine dinucleotide phosphate (NADP; *open bars*) as a cofactor. $^*p < 0.05$.

In vivo dose-response study

These two volunteers (subjects 1 and 6) gave urine samples for the last 4 days of four 7-day study periods. (1) First control period: Each subject collected a 10-hour (7 AM to 5 PM) urine sample daily for 4 days (one subject missed 1 day of sample collection). (2) Low-dose period: Each subject drank 950 to 1060 ml grapefruit juice a day for 7 days and gave 10-hour urine samples for the last 4 days of the 7-day period. (3) High-dose period: Each subject drank 1900 to 2100 ml (double volume of low-dose period) grapefruit juice for 7 days and gave daily 10-hour urine samples for the last 4 days. (4) Second control period: Each subject gave daily 10-hour urine samples for 4 days after 3 days of a washout period.

In vivo metabolic balance study

Protocol. Two different healthy male volunteers (aged 26 and 31 years), not previously screened for responsiveness to grapefruit juice, gave informed written consent and were admitted to the clinical research center for 3 weeks. An evaluation before the study

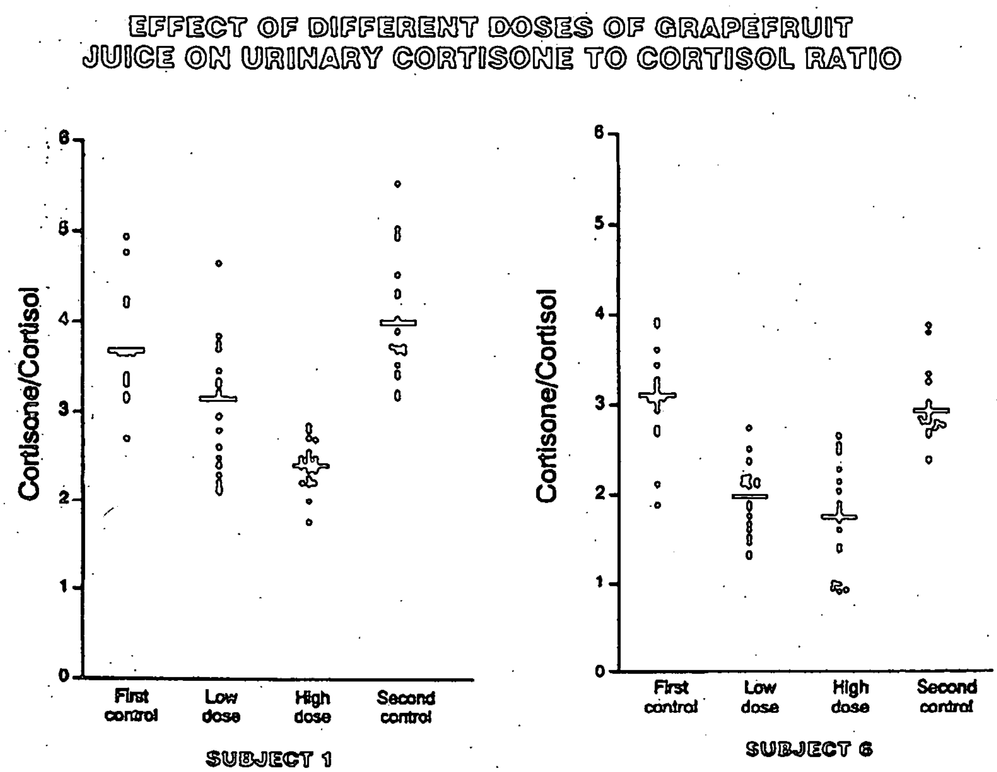


Fig. 3. Urinary cortisone/cortisol ratios in subjects in dose-response study. Each period include four daily urine collections. Each urine sample was assayed twice, each assay in duplicate. Each *point* represents a single assay (four points per daily urine).

showed normal physical findings, serum chemistry, hematology, and ECG for both subjects. They ate a diet that had a constant amount of sodium and potassium during the study (potatoes, bananas, and lemonade during control periods to balance the grapefruit juice during the experimental period). Their blood pressures and body weights were measured daily. Twenty-four-hour urine was collected for free cortisone, cortisol, Na^+, and K^+ for the last 4 days of three 7-day study periods. Blood samples were drawn for Na^+ and K^+ for the same periods. Plasma renin activity, aldosterone and cortisol, and urinary aldosterone excretion were measured at the end of each period. The first and third weeks were the control periods. The second week was the experimental period in which 1500 ml grapefruit juice (100% from concentrate, Ocean Spray Cranberries Inc., Lakeville, Mass.) was consumed daily.

Statistics. The Bonferroni *t* test after a one-way ANOVA was used to assess statistically significant differences. Statistical significance was assumed when the corresponding *p* values were lower than $\alpha = 0.05$.

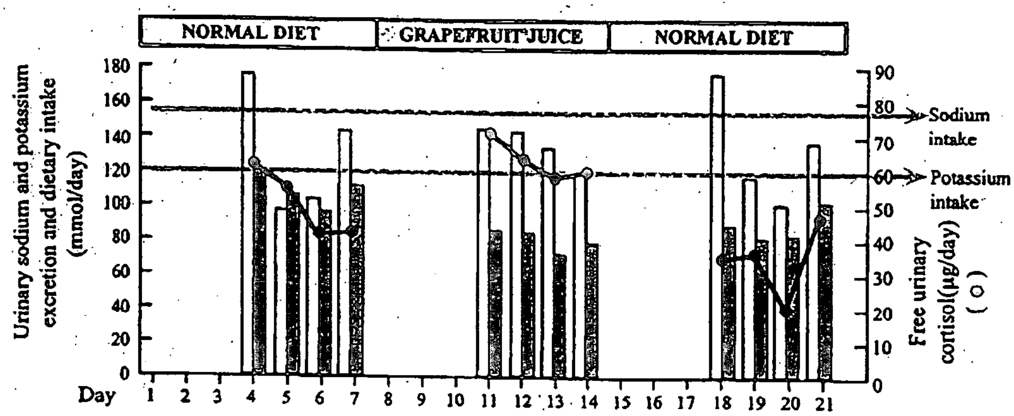
Approval. All human studies were approved by the Cornell Institutional Review Board.

Table I. Inhibition of 11β-OHSD in microsomes of guinea pig kidney by various flavonoids in the presence of NAD or NADP

	IC_{50} (μmol/L)	
Flavonoids	*NAD*	*NADP*
Quercetin*	192 ± 18	355 ± 82
Apigenin*	284 ± 25	125 ± 16
Kaempferol	322 ± 13	293 ± 62
Naringenin*	496 ± 77	264 ± 63
Hesperetin*	769 ± 69	509 ± 45
Naringin*	21,191 ± 4,949	10,550 ± 1,136
Hesperidin	>55,000	>50,000

Data are mean values ± SD.
11β-OHSD, 11β-Hydroxysteroid dehydrogenase; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; IC_{50}, drug concentration that inhibited the enzyme by 50%.
*$p < 0.05$; NAD compared with NADP.

SUBJECT A



Urinary cortisone/cortisol		2.7 2.9 3.1 3.1	1.9 2.2 2.2 2.1	3.5 3.0 3.8 2.8
Plasma cortisol (mcg/dl)	9.5	11.8	12.8	13.5
Plasma renin activity(ng/ml/hr)	0.33	1.4	1.4	1.6

Fig. 4. Values for subject A in metabolic balance study. *Open bars,* Daily urinary sodium excretion; *solid bars,* potassium excretion. The cortisone/cortisol ratios in the grapefruit juice period are significantly different from each normal diet period. The urinary free cortisol during grapefruit juice differs significantly from the first but not the second control period.

RESULTS

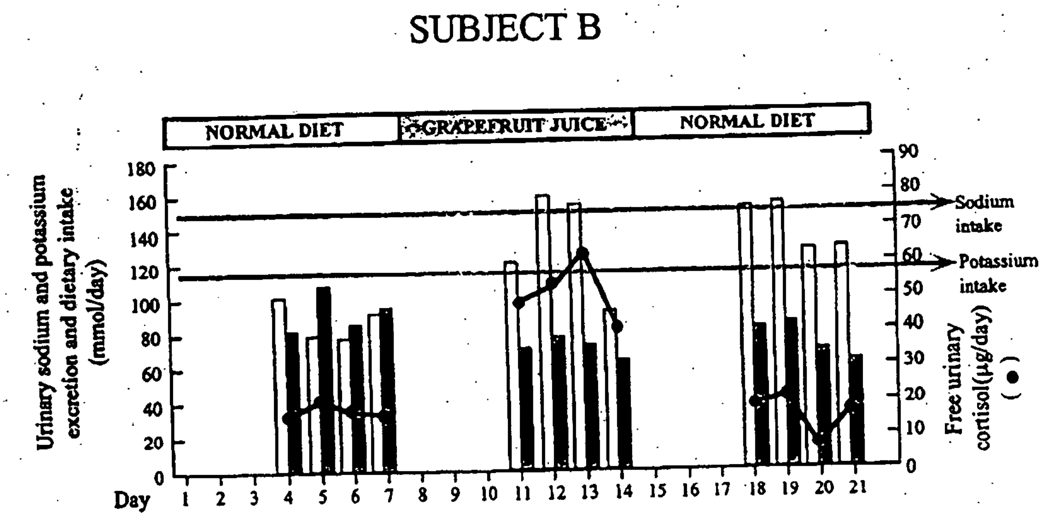
In vitro study

The renal cortex homogenate obtained from male guinea pigs readily converted cortisol to cortisone after 1 hour of incubation at 37° C with NAD or NADP as a cofactor. There was no difference in the conversion rate between NAD and NADP (mean ± SD, 75.1% ± 7.53% with NAD versus 71.0% ± 6.85% with NADP; $p > 0.05$). However, the Michaelis-Menten constant (K_m) values for NAD and NADP calculated from the double reciprocal plots were significantly different (36.4 ± 7.02 µmol/L with NAD versus 57.6 ± 13.1 µmol/L with NADP; $p < 0.05$).

Each flavonoid inhibited the enzyme in a concentration-dependent manner. The inhibition rates for most flavonoids with use of NAD differed from that with use of NADP (Fig. 2). The IC_{50} values of the flavonoids to inhibit the NAD- or NADP-utilizing form of 11β-OHSD are given in Table I. Quercetin was the most potent inhibitor with NAD; apigenin, kaempferol, and naringenin had similar potencies. Apigenin was found to be the most potent inhibitor with NADP, whereas the potency of naringenin, kaempferol, and quercetin were similar. Naringin and hesperidin were poor inhibitors, and their IC_{50} values were much less than that of their aglycons, naringenin and hesperetin. The IC_{50} values of each flavonoid with use of NAD as a cofactor differed from the IC_{50} values with NADP as the cofactor, except for kaempferol.

In vivo dose-response study

The two subjects who drank grapefruit juice showed a dose-dependent decrease in their urinary cortisone/cortisol ratios, indicating inhibition of 11β-OHSD by grapefruit juice (Fig. 3). Each 4-day period was statistically significantly different from the control periods, and the low- and high-dose periods differed in subject 1 statistically and in subject 6 numerically but not statistically.



Urinary cortisone/cortisol		3.7 3.8 3.6 2.3		1.8 1.5 1.6 1.8		3.4 3.8 4.3 3.7	
Plasma cortisol (mcg/dl)	7.1		10.9		9.6		10.0
Plasma renin activity(ng/ml/hr)	1.3		3.5		2.5		2.1

Fig. 5. Values for subject B in metabolic balance study. *Open bars,* Daily urinary sodium excretion; *solid bars,* potassium excretion. The cortisone/cortisol ratios and the urinary free cortisol during the grapefruit juice period are significantly different from both control periods.

In vivo metabolic balance study

The actual values for each subject are presented in Figs. 4 and 5. The mean ratios of the urinary cortisone to cortisol fell with grapefruit juice and recovered during the second control period (mean ± SD, 3.27 ± 0.48 during the first control period, 1.88 ± 0.28 during the grapefruit juice period, and 3.52 ± 0.46 during the second control period). Urinary free cortisol levels also were increased during the grapefruit juice period and returned to the control level after subjects ceased to drink grapefruit juice (mean ± SD, 34.3 ± 19.0 for the first control period, 58.2 ± 9.2 for the grapefruit juice period, and 26.3 ± 12.9 for the second control period; $p < 0.05$ for each control period compared with grapefruit juice period). There was a little change in the body weight during the study (67.3 ± 0.4, 67.8 ± 0.3, and 67.5 ± 0.1 kg for subject A and 73.5 ± 0.8, 74.4 ± 0.2, and 74.4 ± 0.1 kg for subject B, in the first control, grapefruit juice, and second control periods, respectively). The urinary sodium and potassium values were variable during the study. There were no significant changes in plasma potassium levels and blood pressure values during the study.

DISCUSSION

Grapefruit juice is known to inhibit the first-pass oxidation of felodipine and nitrendipine,[19,20] presumably because of compounds in the juice that inhibit cytochrome P450 3A. We did this study to learn if it also inhibited another in vivo oxidation, that of 11β-OHSD. We tested several flavonoids present in grapefruit juice for their ability to inhibit 11β-OHSD from guinea pig renal cortex microsomes. The two different isoforms of the enzyme, NAD-dependent and NADP-dependent 11β-OHSD, had different K_m values for cortisol, and the flavonoids had different IC_{50} values for the two forms. We confirmed

the finding of Walker et al.[11] of similar conversion rates for the two forms.

There are a number of flavonoids in grapefruit juice. Naringin is the most abundant flavonoid, present in concentrations of up to 1 mmol/L.[21] It is thought to be converted to the aglycone naringenin in the intestine after oral administration. Because the flavonoids in grapefruit juice inhibited 11β-OHSD in vitro, we evaluated the ability of grapefruit juice to inhibit the enzyme in vivo. Drinking grapefruit juice lowered the urinary cortisone/cortisol ratios in the two investigators and both inpatient subjects, indicating in vivo inhibition of the enzyme. At the doses consumed, it did not change renal electrolyte clearance. Natural licorice in very high doses causes mineralocorticoid effects by inhibition of this enzyme.[4,22,23] We think that grapefruit juice inhibited 11β-OHSD, but the effect was too mild to cause electrolyte changes in these subjects because their urinary free cortisol did not exceed the normal range. A possible alternative explanation is that ring A reduction of cortisol and not 11β-OHSD inhibition is the major cause of the syndrome of apparent mineralocorticoid excess.[23-26]

If the conventional view that 11β-OHSD inhibition is the cause of the syndrome, and if there are differences in different people's enzyme sensitivity to these inhibitors, as we found with different strains of guinea pigs for gossypol inhibition,[8] some people may increase their potassium clearance if they drink large amounts of grapefruit juice. Furthermore, flavonoids are sold in tablet form in health food stores and drug stores. If people take large quantities of flavonoids as dietary supplements, it is possible that the flavonoids may cause sufficient 11β-OHSD inhibition to produce the syndrome of apparent mineralocorticoid excess.

We thank Patricia Danton for her help.

References

1. Ulick S, Ramirez LC, New MI. An abnormality in steroid reductive metabolism in a hypertensive syndrome. J Clin Endocrinol Metab 1977;44:799-802.
2. Card WI, Mitchell W, Strong JA, Taylor NRW, Tompsett SL, Wilson JMG. Effects of liquorice and its derivatives on salt and water metabolism. Lancet 1953;1:663-8.
3. Borst JCG, ten Holt SP, de Vries LA, Molhuysen JA. Synergistic action of liquorice and cortisone in Addison's and Simmond's disease. Lancet 1953;1:657-63.
4. Farese RV, Biglieri EG, Shackleton CHL, Irony I, Gomez-Fontes R. Licorice-induced hypermineralocorticoidism. N Engl J Med 1991;325:1223-7.
5. Monder C, White PC. 11β hydroxysteroid dehydrogenase. Vitam Horm 1993;47:187-271.
6. Edwards CRW, Walker BR, Benediktsson R, Seckl JR. Congenital and acquired syndromes of apparent mineralocorticoid excess. J Steroid Biochem Mol Biol 1993;45:1-5.
7. Qian SZ, Wang ZG. Gossypol: a potential antifertility agent for males. Annu Rev Pharmacol Toxicol 1984; 24:329-60.
8. Sang GW, Lorenzo BJ, Reidenberg MM. Inhibitory effects ofgossypol on corticosteroid 11β-hydroxysteroid dehydrogenase from guinea pig kidney: a possible mechanism for causing hypokalemia. J Steroid Biochem Mol Biol 1991;39:169-76.
9. Song D, Lorenzo BJ, Reidenberg MM. Inhibition of 11β hydroxysteroid dehydrogenase by gossypol and bioflavonoids. J Lab Clin Med 1992;120:792-7.
10. Zhang YD, Lorenzo BJ, Reidenberg MM. Inhibition of 11β hydroxysteroid dehydrogenase obtained from guinea pig kidney by furosemide, naringenin and some other compounds. J Steroid Biochem Mol Biol 1994;49:81-5.
11. Walker BR, Campbell JC, Williams BC, Edwards CRW. Tissue- specific distribution of the NAD^+-dependent isoform of 11β- hydroxysteroid dehydrogenase. Endocrinology 1992;131:970-2.
12. Mercer WR, Krozowski ZS. Localization of an 11β-hydroxysteroid dehydrogenase activity to the distal nephron. Evidence for the existence of two species of dehydrogenase in the rat kidney. Endocrinology 1992; 130:540-543.
13. Monder C. The forms and functions of 11β-hydroxysteroid dehydrogenase. J Steroid Biochem Mol Biol 1993;45:161-5.
14. Lakshmi V, Nath N, Muneyyirch-Delale O. Characterization of 11β-hydroxysteroid dehydrogenase of human placenta: evidence for the existence of two species of 11β-hydroxysteroid dehydrogenase. J Steroid Biochem Mol Biol 1993;45:391-7.
15. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet 1993;342:1007-11.
16. Brown JP. A review of the genetic effects of naturally occurring flavonoids, anthraquinones, and related compounds. Mutat Res 1980;75:243-77.
17. Ayres PJ, Garrod O, Simpson SA, Tait JF. A method for the determination of aldosterone, cortisol, and corticosterone in biological extracts, particularly applied to human urine. Biochem J 1957; 65:639-46.
18. Peterson RE, Pierce CE, The metabolism of corticosterone in man. J Clin Invest 1960;30:741-57.
19. Bailey DG, Spence JD, Munoz C, Arnold JMO. In-

teraction of citrus juices with felodipine and nifedipine. Lancet 1991;337:268-9.
20. Soons P, Vogels B, Roosemalen N, et al. Grapefruit juice and cimetidine inhibit stereoselective metabolism of nitrendipine in humans. CLIN PHARMACOL THER 1991;50:394-403.
21. Baily DG, Arnold MO, Spence JD. Grapefruit juice and drugs. Clin Pharmacokinet 1994;26:91-8.
22. Stewart PM, Wallace AM, Valentino R, Burt D, Shackleton CH, Edwards CR. Mineralocorticoid activity of liquorice: 11β-hydroxysteroid dehydrogenase deficiency comes of age. Lancet 1987;2:821-4.
23. Epstein MT, Espiner EA, Donald RA, Hughes H, Cowles RJ, Lun S. Licorice raises urinary cortisol in man. J Clin Endocrinol Metab 1978;47:397-400.
24. Ulick S, Tedde R, Wang JZ. Defective ring A reduction of cortisol as the major metabolic error in the syndrome of apparent mineralocorticoid excess. J Clin Endocrinol Metab 1992;74:593-9.
25. Ulick S, Wang JZ, Hanukoglu A, Rösler A. The effect of carbenoxolone on the peripheral metabolism of cortisol in human patients. J Lab Clin Med 1993;22:673-6.
26. Morris DJ. The role of steroid metabolism in protective and specificity conferring mechanisms of mineralocorticoid action. Vitam Horm 1995;50:461-85.

C. N., Wirth, P., Coligan, J. E., Albrechtsen, R., Mudry, M., and Sobel, M. E. (1986). Altered levels of laminin receptor mRNA in various human carcinoma cells that have different abilities to bind laminin. *Proc. Natl. Acad. Sci. U. S. A.* 83, 7137–7141.

Wilke, M. S., and Skubitz, A. P. N. (1991). Human keratinocytes adhere to multiple distinct peptide sequences of laminin. *J. Invest. Dermatol.* 97, 141–146.

Woo, H.-J., Lotz, M. M., Jung, J. V., and Mercurio, A. M. (1991). Carbohydrate-binding protein 35 (Mac-2), a laminin binding lectin, forms functional dimers using cysteine 186. *J. Biol. Chem.* 266, 18419–18422.

Yamada, K. M. (1991). Adhesive recognition sequences. *J. Biol. Chem.* 266, 12809–12812.

Yow, H., Wong, J. M., Chen, H. S., Lee, C., Steele, G. D., and Chen, L. B. (1988). Increased mRNA expression of a laminin binding protein in human colon carcinoma. Complete sequence of a full-length cDNA encoding the protein. *Proc. Natl. Acad. Sci. U. S. A.* 85, 6394–6398.

Yurchenco, P. D., Tsilibary, E. C., Charonis, A. S., and Furthmayr, H. (1985). Laminin polymerization in vitro. *J. Biol. Chem.* 260, 7636–7644.

f.M

VITAMINS AND HORMONES, VOL. 47

11β-Hydroxysteroid Dehydrogenase

CARL MONDER*,[1] AND PERRIN C. WHITE[1]

*The Population Council
Center for Biomedical Research
New York, New York 10021

[1]Department of Pediatrics
Cornell University Medical School
New York, New York 10021

I. Historical Origins

It was experimentally established in 1856 (Brown-Sequard, 1856) that adrenalectomy in animals is fatal, but not until 1927 was it shown

by Rogoff and Stewart (1927) that adrenal extracts could maintain adrenalectomized animals. By 1937, Reichstein in Basel, Switzerland, and Kendall in Rochester, Minnesota, had demonstrated that the active substances in adrenal cortical extracts were steroids. In that year Reichstein and his colleagues synthesized 11-deoxycorticosterone (Steiger and Reichstein, 1937), which, although it was not a quantitatively important secretory product of the adrenal cortex, was used for many years to treat Addisonian patients with some, but by no means total, success. A consensus soon emerged that the therapeutically active adrenal steroid contained oxygen at C-11 (Kendall, 1941; Ingle, 1940; Thorn, 1944; Olson *et al.*, 1944). What complicated discussion of the identity of the hormone of the adrenal cortex was the large number of steroids extracted from slaughterhouse tissue (Fieser and Fieser, 1959). Many of these were 11-oxygenated and were thus candidates for the active steroid. Studies with isolated, perfused adrenals (Reichstein and Shoppee, 1943), analysis of urine from normal individuals (Conn *et al.*, 1951) or patients with Cushing's disease (Mason, 1950; Sprague *et al.*, 1951), and adrenal vein blood (Reich *et al.*, 1950; Nelson *et al.*, 1951; Bush, 1953) led to the conclusion that cortisol (F) and corticosterone (B) are the primary secretory products of the adrenal gland. There was evidence that adrenal enzymes were capable of oxidizing the 11-hydroxy group of corticosterone and cortisol to an 11-keto group (Hechter *et al.*, 1951; Burstein *et al.*, 1953; Fazekas *et al.*, 1970), and that patients treated with cortisone (E) excreted some F (Mason, 1950; Sprague *et al.*, 1951; Burton *et al.*, 1953; Miller and Axelrod, 1953). Although the interconversion of the 11-oxygenated pairs, F and E, and B and 11-dehydrocorticosterone (A) could be readily demonstrated, their biological relationships to each other were not obvious. It was suggested that both cortisol and cortisone were converted to the true active steroid (Hechter *et al.*, 1953). However, cortisone was ineffective when injected into arthritic joints, in contrast with the pronounced antiarthritic effects of the orally administered steroid (Hollander *et al.*, 1951; Zacco *et al.*, 1954). The conclusion that cortisone is biologically inactive and must be converted to its physiologically active form, cortisol, by reduction of the 11-oxo group was supported by other clinical observations (Boland, 1952; Dixon and Bywaters, 1953; Cope and Hurlock, 1954), and by studies with experimental animals (Eisenstein, 1952; Fish *et al.*, 1953; Burton *et al.*, 1953; Caspi *et al.*, 1953; Amelung *et al.*, 1953a; Dobriner, 1951; Savard *et al.*, 1953). In 1953, sufficient quantities of cortisol became available to permit its metabolism to be studied (Fieser and Fieser, 1959). Oral administration of cortisol acetate to human subjects resulted in the

excretion of 11-oxo C_{21} and C_{19} steroids (Burstein *et al.*, 1953). An enzyme responsible for catalyzing the oxidation of cortisol to cortisone was found in rat liver (Amelung *et al.*, 1953a,b) and named "11β-hydroxy dehydrogenase" (Hubener *et al.*, 1956). It is now known as 11β-hydroxysteroid dehydrogenase (11-HSD). Figure 1 illustrates the transformations catalyzed by this enzyme.

II. Distribution, Properties, and Behavior of 11-HSD

A. Tissue Distribution

Catalysis of 11-oxidation and 11-oxoreduction is not uniformly distributed among tissues. In liver, 11-oxoreduction is the dominant activity; in most other tissues, it is 11β-hydroxy oxidation. Whether this behavior is due to the expression of separate enzymes or to the tissue-specific behavior of a unique 11β-hydroxysteroid dehydrogenase was a question first posed 35 years ago (Bush, 1956, 1959; Bush and Mahesh, 1959a). Most investigators have interpreted the results of their studies on steroid metabolism at position 11 in terms of a single enzyme, designated by the Nomenclature Committee of the International Union of Biochemistry as EC 1.1.1.146 (11β-hydroxysteroid:NADP$^+$ 11-oxidoreductase) (Webb, 1984). Within this context, there have been suggestions of multiple enzyme forms, based on the fact that the char-

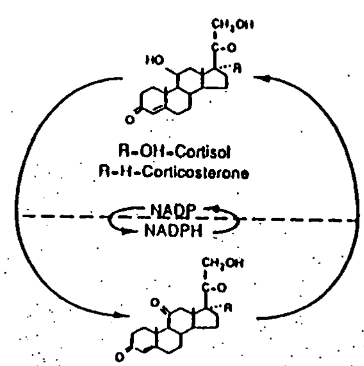


FIG. 1. Transformations catalyzed by 11β-hydroxysteroid dehydrogenase.

acteristics of the enzyme in different tissues varied over a range that was too wide to be accounted for by error or interlaboratory variability.

Table I summarizes our current knowledge of the tissue distribution of 11-HSD. For convenience, activity reported to occur in the oxidative direction is called corticosteroid 11β-dehydrogenase (11-DH), and reductive activity is called corticosteroid 11-oxoreductase (11-OR). From the available literature, it is difficult to evaluate whether 11-DH and 11-OR activities are both present in a particular organ (Fish *et al.*,

TABLE I
DISTRIBUTION OF 11-HSD IN MAMMALIAN TISSUES[a]

Tissue	11β-Dehydrogenase	11-Oxoreductase
Liver	H, D, M, Rb, G, Rt	M, Rb, G, Rt, C
Kidney	H, D, M, Rb, G, Rt	—
Lung	D, M, Rb, G, Rt	H, M, Rb, Rt
Testis	H, Rb, Rt, M	Rt
Brain	D, M, Rt, B	R
Spleen	D, M, G	M
Adrenal cortex	H, D, Rb, Rt, Sh, C, MV	MV
Diaphragm	D, Rt	—
Skeletal muscle	M, Rt, C	—
Blood vessels	Rb, Rt	—
Heart	Rt	—
Lymphocytes	M, Rt	Rt
Thymocytes	M, Rt	M, Rt
Small intestine	H, D, Rt	—
Colon	Rt	—
Placenta	H, Rt, B	H
Ovary	—	—
Uterus	H	H
Myometrium	—	—
Amniotic membrane	H	H
Decidua	H	H
Chorion	H	H
Adipose tissue	H	—
Salivary gland	Rt	—
Mammary gland	H	—
Skin	—	H
Gingival tissue	—	H

[a]H, human; D, dog; M, mouse; Rb, rabbit; G, guinea pig; Rt, rat; C, cattle; B, baboon; MV, meadow vole; Sh, sheep. The table cites positive identification of 11-HSD in the oxidative direction (11β-dehydrogenase) or in the reductase direction (11-oxoreductase) in the investigated species. Absence of measureable activity or no reported activity is indicated by a dash.

1953; Bush *et al.*, 1968; Koerner, 1969; Monder and Lakshmi, 1989a). Where reversibility has been reported, the results have not generally been consistent. The 11-HSD in human adipose tissue has been reported to catalyze only oxidation. Results with intestinal mucosa and skin (Murphy, 1981; Hsia and Hao, 1966; Hammami and Siiteri, 1990; Burton and Anderson, 1983) are in conflict. The reasons for the great differences between laboratories are not clear, but the following sources of variation are probably important; (*a*) the pH of measurement; (*b*) the relative stabilities of the dehydrogenase and oxoreductase activities (Lakshmi and Monder, 1985a); (*c*) incompletely expressed or "latent" enzyme (Lakshmi and Monder, 1985b); (*d*) the age of the animal, its sex and diet, and the possible presence of endogenous inhibitors or activators; (*e*) the developmental stage of the animal (Murphy, 1981); (*f*) substrate specificity (Koerner, 1969).

B. Physiological Functions

It has been suggested that 11-HSD protects cells against the toxic effects of excess corticosteroid (Dougherty *et al.*, 1961; Berliner, 1965). 11β-Dehydrogenase may be a component of a degradation pathway, strategically placed to inactivate corticosteroids prior to their exposure to receptor or to prevent the return of the steroid to receptor. The enzyme also serves a conservationist function, since the oxidized form of the steroid can be reduced by 11-oxoreductase to its active reduced form thus contributing to the circulating cortisol, and providing a buffer against the changes in blood level caused by paroxysmal secretion of the adrenal. The enzyme can thus integrate the availability of corticosteroids to target organs and their metabolism. An overview of these proposed interrelationships is presented in Fig. 2.

C. Enzymatic Properties

1. *Substrate Specificity*

In Tables IIa and IIb are listed all steroids for which oxidation or reduction catalyzed by 11β-hydroxysteroid dehydrogenase have been reported. Table IIc lists the steroids that were found to be neither oxidized nor reduced at C-11. Based on the data in Table II the qualitative effects of substituents on oxidoreduction, i.e., whether steroids carrying the indicated functional group are substrates for 11-HSD, are summarized in Table III.

From the data summarized in Tables II and III it is possible to deduce

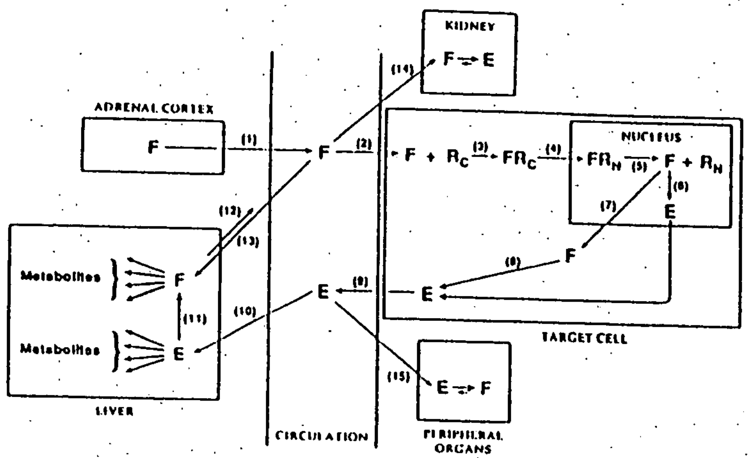


FIG. 2. An overview of the proposed role of 11β-hydroxysteroid dehydrogenase in modulating corticosteroid function. In the model, 11β-hydroxysteroid function and metabolism are illustrated with cortisol (F), and its 11-oxo derivative, cortisone (E). F, synthesized in the adrenal cortex, is transported through the circulation (1) to its target cell (2). The steroid binds to the cytosolic receptor (R_c) (3), which is transported to the nucleus (4), or within the nucleus to the resident receptor (R_N). At an as yet undetermined point in the cell response process the steroid released (5) from the R_N is oxidized to E by nuclear 11-HSD (6), or it leaves the nucleus (7) and is oxidized by microsomal 11-HSD (8). The E thus formed is transported in the circulation (9) to the liver (10) where it is metabolized to inactive end products, or converted to cortisol by 11-oxoreductase (11) which is metabolized or returned to the circulation (12). Circulating cortisol contributes to the liver cortisol pool, as well (13). The kidney is a major contributor to cortisol oxidation (14). Cortisone may be converted to active steroid by peripheral tissues (15) as well as liver; however, evidence for this regenerative pathway is scanty.

how structural changes in steroids bring about corresponding changes in bioactivity. There is considerable evidence that any substituent that inhibits oxidation of the 11β-hydroxy group can enhance corticosteroid potency even if the structural modification does not have an enhancing effect on some other parameter, such as receptor affinity. Bush *et al.* (1968) and Koerner (1969) have analyzed the effects of substituents on oxidation or reduction of 11-oxygenated steroids. From their data, the following conclusions may be drawn: (*a*) A flat A/B junction (5α) is essential for binding to the active site, whereas a buckled A/B junction (5β) prevents binding; (*b*) bulky groups that obstruct the α-surface (2α-

TABLE II
STEROID SUBSTRATES OF 11β-HYDROXYSTEROID DEHYDROGENASE

(a) 11-OH → 11-oxo	
Cortisol	Osinski (1960); Koerner (1969)
Corticosterone	Osinski (1960); Koerner (1969)
11β,17,20β,21-Tetrahydroxypregn-4-en-3-one	Bradlow *et al.* (1968)
11β-Hydroxypregn-4-en-20-one	Koerner (1969); Bush *et al.* (1968)
3α,11β,17,21-Tetrahydroxy-5α-pregnan-20-one	Bush and Mahesh (1959a); Koerner (1969)
11β,17α,20β,21-Tetrahydroxypregn-4-en-3-one	Koerner (1969)
11β,17α,21-Trihydroxy-5α-pregnan-3,20-dione	Koerner (1969)
11β-Hydroxyandrost-4-en-3,17-dione	Koerner (1969)
11β,17,21-Trihydroxy-5α-pregnan-3,20-dione	Koerner (1969)
3β,11β,17,21-Tetrahydroxy-5α-pregnan-20-one	Koerner (1969)
3β,11β,17,21-Tetrahydroxy-pregn-5-en-20-one	Koerner (1969)
11β,17,21-Trihydroxypregn-1,4-diene-3,20-dione	Koerner (1969)
11β,17,20α,21-Tetrahydroxypregn-4-en-3-one	Koerner (1969)
11β,17-Dihydroxypregn-4-ene-3,20-dione	Koerner (1969)
16α-Methyl-cortisol	Bush *et al.* (1968)
16β-Methyl-cortisol	Bush *et al.* (1968)
3α,11β,17,21-Tetrahydroxy-5α-pregnan-20-one-3-acetate	Koerner (1969)
(b) 11-oxo → 11β-OH	
Cortisone	Fish *et al.* (1953); Burton *et al.* (1953)
21-Hydroxypregn-4-en-3,11,20-trione	Mason (1950)
Androst-4-ene-3,11,17-trione	Hubener *et al.* (1956); Bush *et al.* (1968)
Pregn-4-ene-3,11,20-trione	Hubener *et al.* (1956); Bush *et al.* (1968)
17α,20β,21-Trihydroxypregn-4-en-3,11-dione	Koerner (1969); Hubener *et al.* (1956)
17,21-Dihydroxy-pregn-1,4-diene-3,11,20-trione	Bush *et al.* (1968)
17,21-Dihydroxy-5α-pregnan-3,11,20-trione	Bush *et al.* (1968)
9α-Fluorocortisone	Bush *et al.* (1968)
9α-Fluoro-11-oxo-progesterone	Bush *et al.* (1968)
12α-Fluoro-11-oxo-progesterone	Bush *et al.* (1968)
12α-Bromo-11-oxo-progesterone	Bush *et al.* (1968)
3α-Hydroxy-5α-androstane-11,17-dione	Bush *et al.* (1968)
9α-Fluoro-androst-4-en-3,11,17-trione	Bush *et al.* (1968)
9α-Chlorocortisone	Bush *et al.* (1968)
9α-Chloro-androst-4-ene-3,11,17-trione	Bush *et al.* (1968)
12α-Bromo-11-dehydrocorticosterone	Bush *et al.* (1968)
(c) Unreactive steroids	
Tetrahydrocortisol	Hubener *et al.* (1956); Bradlow *et al.* (1968)

(*continued*)

TABLE II (*Continued*)

Tetrahydrocortisone	Hubener *et al.* (1956)
3α-Hydroxy-5β-androstan-11,17-dione	Hubener *et al.* (1956); Bush and Mahesh (1959b)
17α,20β,21-Trihydroxypregn-4-ene-3,11-dione	Bradlow *et al.* (1968)
11β,17α,21-Trihydroxy-5β-pregnan-3-one	Bush *et al.* (1968); Koerner (1969)
2α-Methylcortisone	Bush and Mahesh (1959b)
2α-Methylcortisol	Bush and Mahesh (1959b)
3α,17α,20α,21-Tetrahydroxy-5β-pregnan-11-one	Huebener *et al.* (1956)
3α,17α,20β,21-Tetrahydroxy-5β-pregnan-11-one	Huebener *et al.* (1956)
11β-Hydroxyestrone	Koerner (1969)
12α-Bromo-11-dehydrocorticosterone	Bush *et al.* (1968)
12α-Methyl-11-oxoprogesterone	Bush *et al.* (1968)
9α-Fluorocortisol	Bush *et al.* (1968)
12α-Bromo-11β-hydroxyprogesterone	Bush *et al.* (1968)
12α-Fluorocorticosterone	Bush *et al.* (1968)
12α-Bromocorticosterone	Bush *et al.* (1968)
2α-Methyl-9α-fluorocortisol	Bush *et al.* (1968)
2α-Methyl-9α-fluoro-11β-hydroxyprogesterone	Bush *et al.* (1968)
11β,17,21-Trihydroxy-16α-methyl-pregn-1,4-diene-3,20-dione	Bush *et al.* (1968)
9α-Fluoro-11β,16α,17α,21-tetrahydroxypregn-1,4-diene-3,20-dione	Bush *et al.* (1968)
2α-Methyl-androst-4-ene-3,11,17-trione	Bush *et al.* (1968)
Cortisol-21-sulfate	Koerner (1969)
Cortisol-21-phosphate	Koerner (1969)
Cortisol-21-acetate	Koerner (1969)
Cortisol-21-hemisuccinate	Koerner (1969)
3α-Hydroxy-5β-androstane-11,17-dione	Bush *et al.* (1968)

methyl) inhibit binding; (c) aromatic A ring is forbidden; (*d*) effects of halogens are more likely to be the consequence of their inductive effects than their steric effects; (*e*) steroids with bulky substituents (acetyl, phosphate) at C-21 are not substrates. Structural studies lead to the conclusion that the steroid α-surface binds to the enzyme, and that hydrogen transfer occurs from the 11α-position. Consequently, variations in the velocities of nonhalogenated steroids can be attributed to steric factors.

The inability of 2α-methyl steroids to be oxidized or reduced at C-11 played an important historical role in reinforcing the conclusion that cortisol, and not cortisone, was the active steroid hormone (Bush and Mahesh, 1959b). The metabolically active 2α-methyl-11β-hydroxysteroid could not be oxidized by 11-HSD and the 2α-methyl-11-oxosteroid was inactive as a glucocorticoid, supporting the importance

TABLE III
EFFECTS OF FUNCTIONAL GROUPS ON SUBSTRATE SPECIFICITY OF 11-HSD

Functional group	Oxidation[a]	Reduction[a]
1-one	+	+
2α-Methyl	−	−
3α-Hydroxy	+	NR
3β-Hydroxy	+	NR
Δ^4-3-Oxo	+	+
5β	−	−
5α	+	+
9α-Fluoro	−	+
12α-Fluoro	−	+
16α-Methyl	+	NR
17α-Hydroxy	+	+
20-Hydroxy (α or β)	+	+
21-Methyl	+	+
21-Hydroxy	+	+

[a] +, steroid with indicated functional group is a substrate; −, steroid with indicated functional group is not a substrate. NR, not reported. In evaluating the effects of multiple substituents on substrate specificity, "−" precedes "+." Substituents for which only single examples exist are omitted here, and are listed in Table II.

of the 11β-hydroxy group in glucocorticoid function. These results also helped to disprove the hypothesis that steroids affect metabolism by directly participating as cofactors in transhydrogenation reactions (Williams-Ashman and Liao, 1964).

2. *Steroid Inhibitors*

The catalysis of 11-oxidation is inhibited by a number of structurally diverse steroids, including representatives of the C_{21} and C_{19} series. Inhibitors of reduction have also been shown to include C_{21} and C_{19} steroids, though fewer studies have been performed in this direction. Some C_{18}, C_{19}, and C_{21} steroids inhibit neither oxidation nor reduction. The steroids that have been investigated for their ability to inhibit 11-HSD are listed in Table IV. On the basis of the data, we conclude that inhibition of 11β-dehydrogenase is not caused by the following: 2α-CH_3, 5β-H, 6α-OH, 6β-OH, 12α-OH, 15α-OH, 16α-OH, 20α-OH, 11-oxo, 18-oxo, 16(17)-ene. Steroids devoid of oxygen at C-11 are generally not inhibitors, or inhibit oxidation poorly. The 11α-

TABLE IV
Steroid Inhibitors of 11β-Hydroxysteroid Dehydrogenase

(a) Oxidation (11-OH → 11-oxo)	
C_{21} and C_{19} steroids	
11α,17,21-Trihydroxy-pregn-4-en-3-one (11-epicortisol)	Burton (1965)
11α,17,21-Trihydroxy-pregn-1,4-diene-3-one (11-epiprednisolone)	Burton (1965)
11α-Hydroxypregn-4-en-3-one (11α-hydroxyprogesterone)	Burton (1965); Murphy and Vedady (1982)
17,21-Dihydroxypregn-4-ene-3,11-dione (cortisone)	Bernal *et al.* (1980); Murphy (1979b)
Cortisol 21-acetate	Bernal *et al.* (1980)
Progesterone	Bernal *et al.* (1980); Murphy and Vedady (1982)
1-Dehydro-16α-methyl-9α-fluorohydrocortisone (dexamethasone)	Bernal *et al.* (1980)
11β,17,21-Trihydroxypregn-1,4-dien-3-one (prednisolone)	Bernal *et al.* (1980); Murphy and Vedady (1982)
9α-Fluorocortisol	Bush *et al.* (1968)
3α,11β,17,21-Tetrahydroxy-5α-pregnan-20-one (allotetrahydrocortisol)	Deckx and DeMoor (1966)
3α,11β,17,20β,21-Pentahydroxy-5α-pregnane (allocortol)	Deckx and DeMoor (1966)
11β,17α,21-Trihydroxy-5α-pregnane-3,20-dione (allodehydrocortisol)	Deckx and DeMoor (1966)
11β-Hydroxytestosterone	Monder and Lakshmi (1989a)
11β-Hydroxyandrost-4-ene-3,17-dione	Deckx and DeMoor (1966); Monder and Lakshmi (1989a)
3α,11β,17β-Trihydroxyandrostane	Monder and Lakshmi (1989a)
3β-Hydroxyandrost-5-en-17-one	Deckx and DeMoor (1966)
11β,17β-Dihydroxy-5β-androstan-3-one	Monder and Lakshmi (1989a)
11β,17β-Dihydroxy-5α-androstan-3-one	Monder and Lakshmi (1989a)
(b) Reduction (11-oxo → 11-OH)	
C_{21} and C_{19} steroids	
11-Oxoprogesterone	Tordny *et al.* (1975)
3α,17,21-Trihydroxy-5β-pregnan-3-20-dione (tetrahydrocortisone)	Bernal *et al.* (1980)
21-Hydroxy-pregn-4-ene-3,11,20-trione	Bernal *et al.* (1980)
Androst-4-ene-3,11,20-trione	Deckx and DeMoor (1966)
3β-Hydroxyandrost-5-en-17-one	Deckx and DeMoor (1966)

(continued)

TABLE IV *(Continued)*

(c) Do not inhibit (11β-OH → 11-oxo)	
C_{21} steroids	
21-Hydroxypregn-4-ene-3,20-dione	Murphy and Vedady (1982)
17α,21-Dihydroxypregn-4-ene-3,11-dione	Murphy and Vedady (1982)
11β-Hydroxypregn-4-ene-3,20-dione-21-sulfate	Murphy and Vedady (1982)
11β,17α-Dihydroxypregn-4-ene-3,20-dione-21-sulfate	Murphy and Vedady (1982)
6α-Hydroxypregn-4-ene-3,20-dione	Murphy and Vedady (1982)
12α-Hydroxypregn-4-ene-3,20-dione	Murphy and Vedady (1982)
6β,11β,17α,21-Tetrahydroxypregn-4-ene-3,20-dione	Murphy and Vedady (1982)
11β,21-Dihydroxy-18-oxo-pregn-4-ene-3,20-dione	Murphy and Vedady (1982)
15α-Hydroxypregn-4-en-3-one	Murphy and Vedady (1982)
16α-Hydroxypregn-4-en-3-one	Murphy and Vedady (1982)
3,20-Dioxo-pregn-4,16-diene	Murphy and Vedady (1982)
3α,20α-Dihydroxy-5β-pregnane	Murphy and Vedady (1982)
9α-Fluoro-11β,17α,21-trihydroxy-16β-methylpregn-1,4-diene-3,20-Dione	Murphy and Vedady (1982)
Tetrahydrocortisol	Bernal *et al.* (1980); Deckx and DeMoor (1966)
3α,11β,17,20α,21-Pentahydroxy-5β-pregnane (α-cortol)	Deckx and DeMoor (1966)
3α,11β,17,20β,21-Pentahydroxy-5β-pregnane (β-cortol)	Deckx and DeMoor (1966)
Tetrahydrocortisone	Bernal *et al.* (1980); Deckx and DeMoor (1966)
2α-Methylcortisol	Bush *et al.* (1968)
C_{19} steroids	
3β,11β-Dihydroxy-5α-androstan-17-one	Murphy and Vedady (1982)
3α,11β-Dihydroxy-5α-androstan-17-one	Murphy and Vedady (1982)
3β,11β-Dihydroxy-5β-androstan-17-one	Murphy and Vedady (1982)
3β,11β,16α-Trihydroxyandrost-5-en-17-one	Murphy and Vedady (1982)
3α,11β-Dihydroxy-5β-androstan-17-one	Monder and Lakshmi (1989a); Murphy and Vedady (1982)
3β-Hydroxy-androst-5-en-17-one-3-sulfate	Murphy and Vedady (1982)
Testosterone	Bernal *et al.* (1980)
5α-Dihydrotestosterone	Bernal *et al.* (1980)
3α-Hydroxy-5β-androstan-17	Deckx and DeMoor (1966)
3α-Hydroxy-5α-androstan-17-one	Deckx and DeMoor (1966)
Androstenedione	Deckx and DeMoor (1966)
Androst-4-ene-3,11,17-trione	Deckx and DeMoor (1966)
Dihydroepiandrosterone	Pepe and Albrecht (1984a)
11β-Hydroxy-5β-androstane	Monder and Lakshmi (1989a)
3α,11β-Dihydroxyandrosten-17-one	Monder and Lakshmi (1989a)

(continued)

TABLE IV (Continued)

C_{18}	
Estradiol	Bernal *et al.* (1980); Abramovitz *et al.* (1984)
Estriol	Bernal *et al.* (1980); Abramovitz *et al.* (1984)
Estrone	Abramovitz *et al.* (1984)
(d) Do not inhibit (11-oxo → 11-OH)	
C_{21}	
2α-Methylcortisone	Bush *et al.* (1968)
Cortisol	Bush *et al.* (1968)
20β-Cortol	Bush *et al.* (1968)
20α-Cortol	Bush *et al.* (1968)
3α,11β,17,20β-21-Pentahydroxy-5α-pregnan-20-one (allocortol)	Bush *et al.* (1968)
Cortolone	Bush *et al.* (1968)
C_{19}	
Androst-4-ene-3,17-dione	Bush *et al.* (1968)
3α-Hydroxy-5α-androstan-17-one	Bush *et al.* (1968)
3α-Hydroxy-5β-androstan-17-one	Bush *et al.* (1968)

hydroxysteroids are probably structural analogs and are competitive inhibitors (Burton, 1965; Bernal *et al.*, 1980; Murphy and Vedady, 1981). Although this may also be true for 11β-hydroxysteroids, it is not yet determined which are site-specific structural analogs and which are competitive substrates.

A second important binding site may be the side chain, which requires small bulk at C-21 (—CH_3, —CH_2OH). Introduction of a bulky or charged group at C-21 may diminish the ability of the steroid to act as a substrate, but not as an inhibitor (compare 11β-hydroxypregn-4-ene-3,20-dione and 11β-hydroxypregn-4-ene-3,20-dione-21-sulfate) (Murphy, 1982). Cortisol-21-acetate is a potent inhibitor of decidual dehydrogenase (Bernal *et al.*, 1980), yet is not a substrate (Koerner, 1969).

Since 11β-hydroxy-9α-fluoro compounds are not oxidized by 11β-hydroxysteroid dehydrogenase, inhibition of cortisol oxidation by 9α-fluorocortisol and dexamethasone is due to displacement of the substrate at the active site by homologs in which a negative inductive effect of the halogen stabilizes the 11β-hydroxy group (Bush *et al.*, 1968; Bush and Mahesh, 1959a). Prednisolone is oxidized by 11-HSD (Koerner, 1969) and the reported inhibitory effect (Bernal *et al.*, 1980) is probably due to substrate competition. A similar explanation is applicable to inhibition of cortisol oxidation by corticosterone (Bernal *et al.*, 1980), which is a better substrate for 11-HSD (Engel *et al.*, 1955; Osinski, 1960; Koerner, 1969) than the former.

There are few studies on steroid inhibition of 11-oxoreduction. Most steroids tested (Table IVd) did not affect 11-oxoreductase. Of several that did, none could be shown to have functional groups specially associated with obligatory inhibition. Reduction of an inhibiting steroid at C-20 (tetrahydrocortisone → cortolone) eliminated its inhibitory effect, suggesting a possible orienting role of the side chain. However, since androgens were inhibitors of 11-oxoreductase, the side chain is not essential for binding to the reductase.

The magnitude of the inhibitory effects of steroid analogs differs between tissues. This is illustrated by the data of Bernal *et al.* (1980), who compared the effects of a variety of steroids on placental and decidual microsomes in the oxidative direction. They observed that testosterone, 5α-dihydrotestosterone, and tetrahydrocortisol inhibited the decidual enzyme, but not the placental enzyme. Perhaps the two organs contain distinct species of 11-HSD.

3. *Subcellular Localization*

11β-Hydroxysteroid dehydrogenase has been found in the microsomal fraction of liver (Ghraf *et al.*, 1975a; Hurlock and Talalay, 1959; Koerner, 1969; Bush *et al.*, 1968), kidney (Mahesh and Ulrich, 1960; Ghraf *et al.*, 1975a; Kobayashi *et al.*, 1987), gonads (Ghraf *et al.*, 1975a), placenta (Bernal *et al.*, 1980), spleen (Deckx and DeMoor, 1966), and lung (Nicholas and Lugg, 1982). Cytosol (post-100,000*g* supernatant) and mitochondria were devoid of activity, or when activity was present in these fractions, it was due to contamination with microsomal or nuclear debris. Several investigators have presented evidence for 11-HSD activity in nuclei. Hierholzer and co-workers (Kobayashi *et al.*, 1987; Schulz *et al.*, 1987; Hierholzer *et al.*, 1990a) confirmed the observation of Mahesh and Ulrich (1960) that kidney nuclei contain significant levels of enzyme activity. The K_m values in the experiments of Kobayashi *et al.* (1987) were 2.2×10^{-7} *M* (microsomes) and 2.7×10^{-7} *M* (nuclei), suggesting that these were identical or similar enzymes. Peterson *et al.* (1965) found 11-HSD in the microsomal and nuclear fractions of rat brain. Sakai *et al.* (1992), however, found enzyme activity exclusively in brain and pituitary nuclei. Thus, the location of 11-HSD is not limited to the endoplasmic reticulum and may be distributed between subcellular organelles in a tissue- or cell-specific manner.

4. *Nucleotide Specificity*

The oxidoreductases that catalyze the transformations of steroid molecules are dependent on pyridine nucleotide coenzymes. These enzymes fall into three categories. The 3β-4-ene and 3α-4-ene hydroxysteroid dehydrogenases (Grosso and Unger, 1964) and 21-hydroxysteroid dehydrogenase (Monder and White, 1963, 1965) have dual nucleotide specificity, utilizing either NAD or NADP as cofactors; guinea pig liver and kidney 17β-hydroxysteroid dehydrogenases exist as particulate NAD-dependent and soluble NADP-dependent forms (Endahl *et al.*, 1960; Endahl and Kochakian, 1962); rat ovarian 20α-hydroxysteroid dehydrogenase (Wiest and Wilcox, 1961) and liver 5β-4-ene reductase (Tomkins and Isselbacher, 1954) are strictly NADP-dependent.

A survey of nucleotide specificity of 11-HSD in various tissues is presented in Table V. Rat liver enzyme has been reported to have dual nucleotide specificity, with NADP more effective than NAD (Hurlock and Talalay, 1959; Bush *et al.*, 1968; Koerner, 1966, 1969). In other tissues, including lung, kidney, placenta, intestinal mucosa, adipose tissue, striated muscle, and spleen, NADP was more effective than NAD, or NAD was not a cofactor. Two groups found that NAD and NADP were equally effective with enzyme from rat kidney (Mahesh and Ulrich, 1960) and human placenta (Meigs and Engel, 1961). Mercer and Krozowski (1992) have proposed that rat kidney contains distinct NADP- and NAD-dependent forms of 11-HSD. NAD was reported to be a better cofactor than NADP with 11β-dehydrogenase from rat mandibular gland. In one study with rat liver, NADPH was a better cofactor than NADH (Bush *et al.*, 1968). The data are consistent with a heterogeneous distribution of NAD- and NADP-responsive forms of 11-HSD.

5. *Kinetic Constants*

The K_m values summarized in Table VI are taken from a variety of sources and extend over a 1000-fold range in the oxidative direction, extending from 0.1 μM for mouse spleen microsomes (Deckx and DeMoor, 1966) to 172 μM for mouse liver microsomes (Burton, 1965). Direct comparison of the various values cannot be readily made, because of the large number of variables that must be taken into consideration: steroid substrate, pH, temperature of incubation, tissue preparation, tissue fraction, cofactor concentration. The broad variability persists even if only microsomes measured at 37°C and pH 7.4 are compared (Burton, 1965; Bernal *et al.*, 1980). The K_m values for cortisone reduction also extend over a wide range.

TABLE V
COENZYME SPECIFICITY OF 11-HSD

Tissue	F → E[a]	E → F[a]	Citation[b]
Rat liver	NADP (NAD not tried)	—	(1)
	NADP > NAD	NADPH > NADH	(2)
	NADP > NAD	—	(3–5)
Rat lung	NADP (NADH not tried)	NADPH (NAD not tried)	(6)
	NADP (NAD inactive)	—[c]	(4)
Rat kidney	NADP = NAD	—[c]	(7)
	NADP > NAD	—[c]	(4,8)
Human placenta	NADP = NAD		(9)
	NADP > NAD	—[c]	(10)
Mouse striated muscle	NADP (NAD inactive)	—[c]	(11)
Bovine striated muscle	NADP (NAD inactive)	—[c]	(11)
Human intestinal mucosa	NADP (NAD not tried)	—[c]	(12)
Human adipose	NADP > NAD	—[c]	(13)
Rat submandibular Gland	NAD > NADP	Not tried	(14,15)
Rat spleen	NADP > NAD	NADPH (NADH not tried)	(16)

[a] F, cortisol; E, cortisone.
[b] (1) Koerner (1969); (2) Bush *et al.* (1968); (3) Hurlock and Talalay (1959); (4) Koerner (1966); (5) Koerner and Hellman (1964); (6) Nicholas and Lugg (1982); (7) Mahesh and Ulrich (1960); (8) Kobayashi *et al.* (1987); (9) Meigs and Engel (1961); (10) Osinski (1960); (11) Sweat and Bryson (1960); (12) Burton and Anderson (1983); (13) Weidenfeld *et al.* (1982); (14) Hoyer and Moller (1977); (15) Furguson and MacPhee (1975); (16) Deckx and DeMoor (1966).
[c] NADPH had little or no effect in the reductive direction.

6. *pH Optimum*

The recorded values for the pH optimum of 11β-hydroxysteroid dehydrogenase, like the kinetic constants, vary broadly when measured in the oxidative or reductive directions. Oxidation of cortisol to cortisone by microsomes of mature rat liver was optimal at about pH 10 (Bush *et al.*, 1968; Koerner, 1969). Fetal mouse liver had a reported pH optimum of 8 (Michaud and Burton, 1977). The value for salivary gland homogenate was pH 7.6 (Furguson and MacPhee, 1975). Human placenta homogenate was reported by one laboratory to optimally oxidize cortisol in the pH range 8 to 9 (Osinski, 1960). Another laboratory reported a maximum above pH 10, with a plateau between pH 7 and 8

TABLE VI
MICHAELIS CONSTANTS REPORTED FOR 11-HSD IN VARIOUS TISSUES

Tissue	Fraction[a]	Variable substrate[b]	pH	K_m (μM)	Citation[c]
Rat liver	mc	Cortisol	7.4	16.4	(1)
Guinea pig liver	mc	Cortisol	7.4	27.1	(1)
Rat liver	mc	Cortisol	7.4	30	(2)
Rat liver	mc	Cortisol	8.6	17.6	(3)
Rat liver	mc	Corticosterone	8.5	9.2	(3)
Rat liver	mc	Corticosterone	8.5	0.22	(4)
Rat liver	nc	Corticosterone	8.5	0.27	(4)
Mouse liver	mc	Cortisol	7.4	172	(5)
Mouse fetal liver	mt	Cortisol	8.0	10	(6)
Rat lung	hom	Cortisol	7.4	4.1	(7)
Rat lung	mc	Cortisol	7.4	1.7	(7)
Mouse spleen	mc	Corticosterone	10	0.11	(8)
Mouse spleen	mc	Cortisol	10	0.23	(8)
Human adipose	hom	Cortisol	7.2	0.5	(9)
Human placenta	Mince	Cortisol	7.4	3.0	(10)
Human placenta	mc	Cortisol	7.4	0.3	(11)
Human decidua	mc	Cortisol	7.4	3.2	(11)

[a] mc, microsomal fraction; nc, nuclear fraction; mt, mitochondrial fraction; hom, homogenate.

[b] Constant cosubstrate was NADP.

[c] (1) Bush *et al.* (1968); (2) Koerner and Hellman (1964); (3) Monder and Lakshmi (1989a); (4) Murphy (1979b); (5) Burton (1965); (6) Michaud and Burton (1977); (7) Nicholas and Lugg (1982); (8) Deckx and DeMoor (1966); (9) Weidenfeld *et al.* (1982); (10) Kobayashi *et al.* (1987); (11) Bernal *et al.* (1980).

(Bernal *et al.*, 1980). Spleen microsomes were maximally effective at about pH 10 (Deckx and DeMoor, 1966). In the reverse direction, few values were available. The range was nevertheless broad, embracing values from pH 6.5 to 7.0 (Michaud and Burton, 1977; Deckx and DeMoor, 1966; Bush *et al.*, 1968).

The cause of such a wide range of values is not immediately apparent. That the method of preparation of the tissue may have played a role is suggested by data reported by Monder and Lakshmi (1989a). Freshly prepared rat liver microsomes generated a pH-activity profile with a maximum at pH 10 similar to what was reported by most investigators (Bush *et al.*, 1968; Koerner, 1969; Koerner and Hellman, 1964; Deckx and DeMoor, 1966). When briefly exposed to detergent, a profile resembling that obtained by Bernal *et al.* (1980) was obtained, with a plateau between pH 7 and 8, and a maximum at more alkaline values.

Varying conditions yielded distinctive pH-activity curves that were less a reflection of the intrinsic property of the enzyme than a composite reflection of the environment of the enzyme and its prior treatment.

D. EFFECTS OF HORMONES

1. *Androgens and Estrogens*

In rats, there is a sex-dependent difference in 11-HSD of liver (Lax *et al.*, 1978, 1979) and kidney that favors males (Smith and Funder, 1991). Consistent with this observation is the strong evidence that 11-HSD is affected by the administration or withdrawal of sex steroids. The reduction of cortisone to cortisol by male rat lung is diminished after castration (Nicholas and Lugg, 1982). Gonadectomy may decrease liver 11-HSD in male rats as well, but appears to have no effect on female rats (Lax *et al.*, 1979). The introduction of testosterone to gonadectomized males is reported to bring the liver enzyme up to normal, whereas estradiol almost completely suppresses liver activity in male and female rats. Testosterone can increase the activity of female liver to about the level of normal male liver (Lax *et al.*, 1979). The response of different organs may not, however, be uniform. It has been reported that 11-HSD in genital skin fibroblasts of squirrel monkey is inhibited by testosterone (Hammami and Siiteri, 1990). These studies suggest that male and female steroids have opposite effects on 11-HSD expression.

The effects of estradiol and testosterone on liver 11-HSD of hypophysectomized rats are different from their effects on gonadectomized animals. Inhibition of activity by estradiol is suppressed, whereas testosterone raises the level of activity somewhat above normal. Hypophysectomy appears to release an endogenous suppression in females, raising the activity above that of comparable male rats. The effects of hypophysectomy are complicated, since this process eliminates numerous peptide and steroid hormones. In general, ablation of the pituitary results in loss of sex steroid dependence of liver enzymes of steroid metabolism (Gustafsson and Stenberg, 1976). The effects have been attributed to growth hormone. However, no studies have yet been performed on the growth hormone dependence of 11-HSD.

The response of neonatal rat testis to androgen and estrogen administration was similar to that seen in livers of hypophysectomized rats. Estradiol lowered 11-HSD activity and testosterone had no effect (Ghraf *et al.*, 1975b). It would therefore be expected that differences in

the level of 11-HSD would be seen in the two sexes in tissues that are responsive to sex steroids. The administration of the antiestrogen MER-25 to pregnant baboons prevented the increase in the capability of the placenta to oxidize cortisol to cortisone with advancing gestation, indicating that estrogen regulates the activity or synthesis of placental 11-HSD (Pepe and Albrecht, 1987). This observation was tested directly by increasing placental estrogen production and showing a similar increase in the extent of oxidation of cortisol to cortisone (Pepe *et al.*, 1988; Baggia *et al.*, 1990).

In the rat kidney, the effects of gonadectomy are unclear. In one study (Ghraf *et al.*, 1975b), it was found that female animals respond to ovariectomy by developing normal male 11-HSD levels, whereas male animals subjected to castration retained the activity unchanged. Hypophysectomy established normal male activity in both sexes. For female animals, therefore, hypophysectomy is equivalent to gonadectomy. In another study (Smith and Funder, 1991), the opposite was found. Gonadectomy decreased renal 11-HSD in males and had no effect on females.

Differences in 11-HSD activity in the two sexes are increased after puberty in normal rats. In liver and kidney, the female values are lower, because of the suppressive effect of estradiol. Values for the gonads, where concentrations of the sex steroids are predictably high, are consistent with those for other tissues (Hoff *et al.*, 1973).

In perfused male rat lung, castration decreased reduction of cortisone to cortisol (Nicholas and Lugg, 1982). Therefore the effects of sex steroids on 11-HSD are broad ranging and affect the enzyme in several organs. Adrenal 11-HSD measured in the oxidative direction is highest in the meadow vole during the winter, and is depressed during the breeding season, which is associated with an increase in corticosterone and increased adrenal size. It has been suggested that the circumannual effect is caused by seasonal ingestion of phytoestrogens (Unger *et al.*, 1978).

2. *Corticosteroids*

Glucocorticoids may intervene in their own metabolism by influencing the activity of 11-HSD. Some indirect suggestion that glucocorticoids affect lung 11-HSD is based on the observation that stress increases the activity of rat lung 11-HSD in the reductive direction (Nicholas and Lugg, 1982). Cortisol, which regulates the mitosis, maturation, and mortality of lymphocytes, also affects the level and direction of their 11-HSD activity. Thymic cells of mice pretreated with cortisol for 9 days showed increased activity in the oxidative direction and no change in the reductive direction (Dougherty *et al.*, 1960). Placental 11-HSD of the baboon may be resistant to corticosteroid hormones. Serum cortisone did not alter the level of placental 11-oxoreductive activity and possibly decreased 11β-dehydrogenase (Pepe and Albrecht, 1985a). Although it is unlikely that the effect is due to direct inhibition by cortisone of enzyme activity, the addition of pregnenolone (250 n*M*), progesterone (25 n*M*) or cortisone (250 n*M*) to human or baboon placental homogenates inhibited oxidation of cortisol to cortisone (Pepe and Albrecht, 1984a). Bernal *et al.* (1982) found no changes in human placental 11-HSD taken after elective cesarean section, or after spontaneous and induced labor. However, injections of dexamethasone into pregnant rhesus monkeys increased cortisol-to-cortisone conversion by the placenta (Althaus *et al.*, 1982).

Isolated perfused fetal rabbit lung oxidized cortisol, but the reverse reaction was minimal (Torday *et al.*, 1976). Lugg and Nicholas (1978) found that the development of 11-HSD in the lung of the fetal rabbit *in vivo* is not affected by dexamethasone after direct injection into the fetus. Fetal rat lung, however, responded to betamethasone, another glucocorticoid analog, with an increase in 11-HSD reduction. *In vitro*, cortisol stimulated growth of fetal human lung cells. This corresponded with increased net conversion of cortisone to cortisol (Smith *et al.*, 1973). Abramovitz *et al.* (1982) showed that fetal lung cells diverged during growth in tissue culture into populations of epithelial cells and fibroblast-like cells which could be cultured separately. The former preferentially oxidized cortisol to cortisone; the latter, which was the dominant surviving cell type, reduced cortisone to cortisol. These findings may explain an apparent contradiction between findings of Smith *et al.* (1973) described above and those of Pasqualini *et al.* (1970a) and Murphy (1978), who found that fetal lung primarily catalyzed cortisol oxidation, as did all other fetal tissues. Reduction of cortisone increased with lung maturity. This developmental pattern also applies for the fetal rat (Smith, 1978). The reduction of cortisone by fetal rabbit lung was reported by Giannopoulos (1974). Mature rat lung rapidly reduced cortisone to cortisol (Nicholas and Kim, 1975). The suggestion that the direction of 11-oxygen metabolism in lung is determined under physiological conditions by the nature of the cell population remains to be tested.

3. *Thyroid*

There have been several studies published on the effects of thyroid hormone on 11-HSD. Species specificity has been observed on the effects of thyroxine on the oxidation of cortisol to cortisone by liver.

Thyroxine administration causes a decrease in male rat liver (Koerner and Hellman, 1964; Lax *et al.*, 1979) that is only apparent after 7 days of continuous exposure. In contrast, hyperthyroid humans respond with increased hepatic activity (Zumoff *et al.*, 1983; Hellman *et al.*, 1961; Gordon and Southren, 1977). Thyroidectomy or hypothyroidism reverses the response, resulting in increased activity in the rat (Koerner and Hellman, 1964) and decreased activity in humans (Zumoff *et al.*, 1983; Hellman *et al.*, 1961; Gordon and Southren, 1977). The effects of thyroid hormones are tissue specific. No changes occur in the kidney (Koerner and Hellman, 1964; Lax *et al.*, 1979; Smith and Funder, 1991) or in the reticuloendothelial system (Dougherty *et al.*, 1960). The proposal that thyroid hormones act by controlling the availability of pyridine nucleotides (Dougherty *et al.*, 1960) appears not to be borne out by experiment (Koerner and Hellman, 1964). Enzyme inhibitors are not formed (Koerner and Hellman, 1964). Whether thyroid hormones directly affect the level of enzyme is not known. It has been suggested that thyroid hormone controls the level of available testosterone, and thus indirectly influences 11-HSD.

4. *Other Hormones*

The activity of placental 11-HSD, which shows activity almost entirely in the oxidative direction, is not affected by prolactin, hCG, or ACTH *in vitro*. The cortisol and cortisone content of amniotic fluid of diabetic and nondiabetic women are identical. Therefore insulin, glucagon, and the various diabetogenic factors do not influence 11-HSD (Baird and Bush, 1960).

III. Developmental Biology and 11-HSD

A. Fetal Development

1. *Placental 11-HSD*

The level of active corticosteroid to which the fetus is exposed is crucial to its development and maturation. Too high exposure can lead to developmental disturbances. The placenta catalyzes the oxidation of the 11β-hydroxy groups of corticosteroids, both natural (Burton and Jeyes, 1968; Bernal and Craft, 1981; Giannopoulos *et al.*, 1982; Pasqualini *et al.*, 1970a; Waddell *et al.*, 1988) and synthetic (Levitz *et al.*, 1978), and thus provides a barrier to the transfer of active glucocorticoid to the fetus by converting the steroids to the biologically inactive 11-oxo form. In keeping with this role, reduction of 11-oxosteroids by placental 11-HSD is low or not detectable (Osinski, 1960; Bernal *et al.*, 1980; Murphy *et al.*, 1974; Murphy, 1979b; Kittinger, 1974). As a consequence of this overwhelming oxidative activity, relatively high proportions of 11-oxocorticosteroid metabolites appear in normal amniotic fluid and fetal cord serum (Osinski, 1960; Baird and Bush, 1960; Bro-Rasmussen *et al.*, 1962). When the placental barrier is bypassed, intraamniotically administered cortisol is absorbed by the human fetus and is oxidized in individual organs slowly, resulting in long-term retention of active steroid (Murphy and Vedady, 1982). The proportion of cortisol relative to cortisone is greater in the amniotic fluid than the cord fluid and rises with gestation in humans (Murphy, 1977a). Chorionic membrane catalyzes a reduction of cortisone to cortisol (Murphy, 1977b; Bernal *et al.*, 1980) and may contribute to the rise in active steroid. Although the direction of metabolism of the 11-oxygen by placenta and chorion does not change during gestation, it is not known whether the levels of 11-HSD activity in these organs change. The few studies that have been performed with human placenta have yielded conflicting results. Giannopoulos *et al.* (1982) have reported quantitative changes in the capacity of placenta and decidua to interconvert cortisol and cortisone; Bernal *et al.* (1982) find no changes in placental corticosteroid metabolism during the terminal stages of pregnancy in the human, but find changes over the longer term. These results may not in fact be contradictory since the span of gestation studied in the two investigations were quite different, the former extending through the major part of pregnancy, and the latter considering only the terminal stages. Tanswell *et al.* (1977) have suggested that reductive capacity of the chorion is valuable for the fetus, because it represents a mechanism for regenerating cortisol for the fetus, possibly acting as an accessory adrenal gland.

2. *The Feto-Placental Unit*

The behavior of the placenta *in vitro* confirms that a highly effective barrier exists against the transfer of 11β-hydroxysteroids from mother to fetus. The ability of the human and primate feto-placental unit to efficiently oxidize cortisol to cortisone results in the transfer of little or no cortisol into the fetus (Althaus *et al.*, 1982), who is thus protected against the teratogenic actions of cortisol (Murphy *et al.*, 1974; Munck and Leung, 1977; Slikker *et al.*, 1982). The existence of this barrier also permits the fetus to retain autonomy over its own cortisol production (Murphy and Branchaud, 1983; Beitins *et al.*, 1972; Mitchell *et al.*, 1981, 1982). The timing of the increase in active corticosteroid level in

the maturing fetus is essential for creating an internal environment compatible with independent life (Murphy, 1977a). Synthetic steroids that are poor substrates for 11-HSD, such as dexamethasone, are transferred to the fetus largely unoxidized (Althaus *et al.*, 1982; Funkenhouser *et al.*, 1978; Anderson *et al.*, 1979). This process may have important pharmacological implications. Pepe and co-workers have studied the transplacental regulation of cortisol metabolism in pregnant old world monkeys. They have suggested that transuteroplacental corticosteroid metabolism may play an important role in the maturation of the pituitary–adrenocortical axis (Pepe and Albrecht, 1985b). Although transfer of cortisone (from maternal cortisol) to the fetus is extensive, little cortisone is converted to the active hormone (Mitchell *et al.*, 1982). Most of the cortisol available to the late gestation fetus, as illustrated with baboon and rhesus, is endogenous (Mitchell *et al.*, 1981; Althaus *et al.*, 1982; Pepe and Albrecht, 1984b), resulting from secretion of the maturing fetal adrenal.

3. *Fetal 11-HSD*

Fetal tissues contribute to the net oxidation of corticosteroids in the developing organism. Brain, gut, liver, and lung in the fetal mouse are all strongly oxidizing at 14 days of gestation. By 19 days, with birth approaching, the tissues show increasing capacity for reduction (Tye and Burton, 1980), in some cases evolving from net oxidation to net reduction. The capacity of the mouse liver to catalyze net reduction continues to increase after birth (Burton and Jeyes, 1968).

Other organs change their relative preference of direction of 11-oxidoreduction during development. The nonpregnant human uterus preferentially oxidizes cortisol to cortisone, but catalyzes the reverse process during early pregnancy (Murphy, 1977b). The net effect of the metabolic events catalyzed by 11-HSD in the fetus is the oxidation of cortisol to cortisone or corticosterone to 11-dehydrocorticosterone (Murphy, 1981; Pasqualini *et al.*, 1970a,b). The proportion of oxidized to reduced form decreases during gestation as 11-HSD in the liver (Michaud and Burton, 1977; Smith *et al.*, 1982) and lung (Nicholas and Lugg, 1982; Smith *et al.*, 1982; Smith, 1978) plays an increasingly important role in reducing the 11-oxo group of the steroid.

The changes in steroid oxidoreduction in the individual fetal organs are intimately connected with the maturational events that prepare the organism for birth and permit its subsequent independent existence (Liggins, 1976). Fetal lung has been the subject of intense study. Pulmonary differentiation is dependent on and accelerated by 11β-hydroxylated corticoids. Glucocorticoids induce synthesis and release of surfactant and the differentiation of alveolar cells (Avery, 1975). Steroid effects on lung maturation have important clinical applications in the prevention or reversal of hyaline membrane disease (Liggins and Howie, 1972). Since the dominant metabolic transformation of glucocorticoids in the fetus is 11-oxidation, the ability of the lung to catalyze 11-oxoreduction is of particular significance. Conversion of cortisone or 11-dehydrocorticosterone to their respective 11-reduced forms is essential for lung differentiation (Torday, 1980; Giannopoulos, 1974; Murphy, 1981; Torday *et al.*, 1976; Drafta *et al.*, 1975) based on the criteria of lung size (Drafta *et al.*, 1975), cell growth (Torday, 1980; Smith *et al.*, 1973), phosphatidylcholine production (Drafta *et al.*, 1975; Torday *et al.*, 1976), and glycogen content (Smith *et al.*, 1982). The ability of fetal lung to reduce 11-dehydrocorticosteroids increases during gestation in rabbit (Dougherty *et al.*, 1960; Torday *et al.*, 1976; but see Hummelink and Ballard, 1986), human (Smith *et al.*, 1973), mouse (Burton and Turnell, 1968), and rat (Smith *et al.*, 1982). It is possible that in human lung the increase in reductive ability during gestation may in part be due to a large decrease in the 11β-dehydrogenase as well as an absolute increase in 11-oxoreductase. It has also been suggested that the driving force in the increase in 11-oxoreduction is an increase in NADPH (Torday *et al.*, 1976).

During the second trimester and early third trimester of pregnancy, 11-oxo steroids exceed 11-hydroxysteroids in the fetal circulation (Waddell *et al.*, 1988; Murphy and Diez d'Aux, 1972; Burton and Jeyes, 1968; Sowell *et al.*, 1971). Murphy *et al.* have documented the extensive catabolism of cortisol to cortisone in the human midterm fetus (Murphy, 1979b, 1981; Murphy and Branchaud, 1983). The magnitude of oxidation of cortisol and corticosterone is dependent on the combined metabolic actions of the placenta, its associated membranes, and the fetal tissues. The relative oxidative and reductive activities in many tissues change with time. In most tissues, irrespective of species, the oxidation of steroids dominates at midgestation. In late gestation, reductase activity is expressed in some tissues. It is not yet known whether the shifts in dehydrogenase–oxoreductase capabilities of some tissues are species specific, nor is it known for most organs when, during development, the expression of 11-HSD activity first appears.

Perinatal reduction is dominated by the lung and liver. In mouse liver net 11-reduction continues to increase after birth. The relative capabilities of each tissue to catalyze 11-oxidation or 11-reduction correlate well with the proportion of 11-oxo- to 11-hydroxysteroids in these tissues (Smith, 1978; Smith *et al.*, 1982). The ratio of reduced to oxidized steroid in tissues at critical stages of development may pro-

vide important clues to determining key molecular events necessary to complete development. There is no doubt that the presence of the physiologically appropriate level of active corticosteroid at the correct stage of development of fetal organs is essential for their proper development and function. In view of this and the known teratogenic effects of corticosteroids when administered in excess to experimental animals, there is ample reason to suspect that 11-HSD performs a crucial mediating role in fetal development.

B. Postnatal Development

1. *In Vivo Metabolism of Corticosteroids*

After birth, overall corticosteroid metabolism at C-11 is reductive. In some organs, such as uterus, parotid gland, colon, and kidney, metabolism continues to be predominantly oxidative. One consequence of the concurrent selective exposure of steroids to oxidative or reductive conditions in the various organs is the excretion of a mix of 11-oxo- and 11β-hydroxysteroid metabolites. In humans and primates, who excrete corticosteroid metabolites mainly by way of the kidney, measurement of urinary steroid metabolites provides an accurate reflection of the oxidoreductive balance. In other organisms, such as rats and mice, that utilize the gastrointestinal tract as the dominant excretory pathway for steroids, establishing the net balance of oxidation and reduction is far more difficult, and has not yet been successfully accomplished.

One approach to the study of murine steroid metabolism utilizes biliary steroids. In rats, about 90% of corticosterone metabolites are recovered from bile (Gustafsson and Gustafsson, 1974), reflecting primarily hepatic metabolism (Eriksson and Gustafsson, 1971). Most identified metabolites contain the 11β-hydroxy group, suggesting that liver metabolism at C-11 is primarily reductive *in vivo*.

In humans, the metabolites of endogenously produced cortisol are excreted into the urine as a mixture of products at different levels of reduction and oxidation (Peterson *et al.*, 1955). These include metabolites reduced in ring A (tetrahydrocortisol, tetrahydrocortisone), and ring A-reduced metabolites further reduced at C-20 (cortols, cortolones); of those metabolites in which oxidation dominates, the major examples are the cortoic acids, C_{21} steroids containing a carboxylic acid group at C-21 (Monder and Bradlow, 1980). There are additionally significant amounts of metabolites resulting from the loss of the ketol side chain, and a number of minor metabolites.

2. *Corticosteroid Metabolites in Health and Disease*

Cortisol in normal humans, male and female, has a biological half-life of 95 to 130 min (Peterson *et al.*, 1955). Cortisone has an average plasma biological half-life of 28 min. This is due in part to its rapid conversion to cortisol, and in part to the greater susceptibility of cortisone to catabolism. Its reduction to cortisol, mediated by 11-HSD, is the basis for its pharmacological action; the leaking away of cortisone by competing catabolic reactions is the reason that cortisone is a less potent pharmacological agent than cortisol. That cortisol is oxidized at C-11, i.e., that the oxidation–reduction process is physiologically freely reversible, is revealed by the profile or urinary metabolites. The ratio of the major metabolic products tetrahydrocortisol (THF), allotetrahydrocortisol (5α-THF, ATHF) and tetrahydrocortisone (THE), expressed as (THF + ATHF)/(THE) = R, has been used as a measure of the physiological oxidoreductase activity at C-11. Numerous studies have confirmed that alterations in physiological status cause significant changes in this ratio. Table VII presents a qualitative assessment of the effects of a variety of conditions on the value of R relative to that of normal subjects, whose values range from 0.5 to 2. Irrespective of the nature of the illness, the proportion of 11-reduced metabolites increased relative to control (presumably normal) populations, with few exceptions. The changes were not large, rarely exceeding 50%, and are insufficiently great in magnitude to distinguish whether changes in the level of enzyme or pyridine nucleotide are rate limiting, or whether the difference can be attributed to a selective redistribution of 11-oxosteroids between tetrahydro and pentahydro metabolites (Bradlow *et al.*, 1968; Zumoff *et al.*, 1968b). Zumoff *et al.* have shown that while (THF + ATHF)/(THE) increased in cirrhosis (Zumoff *et al.*, 1967) the total value of C-11 hydroxy/C-11-oxo, including all urinary steroids, was unchanged from normal (Zumoff *et al.*, 1968a), suggesting increased conversion of THE to cortolones. Where the secretion of cortisol from the adrenal results in peripheral accumulation great enough to exceed the ability of the organism to dehydrogenate at C-11, the urinary cortisol/cortisone and THF/THE ratios increase (Baulieu and Jayle, 1957).

During postnatal development, the R values change from ca.0.1 at birth to approximately unity, as Fig. 3 shows. The early low values of this ratio are the consequence of the fact that in the recently born infant, 11-dehydrogenation is highly active, resulting in the excretion of THE, but little THF. This pattern also occurs in primates other than human (Pepe and Townsley, 1976). The proportion of THE and THF

TABLE VII
EFFECT OF DISEASE ON THE PROPORTION OF URINARY 11β-HYDROXY TO 11-OXO METABOLITES

Condition	Effect[a]	Citation[b]
Cushing's disease or ACTH	$R_C > R_N$	(1–5)
Infection, nonspecific illness	$R_C > R_N$	(6,7)
Rheumatic disorders	$R_C > R_N$	(7,8)
Cirrhosis	$R_C > R_N$	(9)
Essential hypertension	$R_C > R_N$	(10,11)
Chronic myelogenous leukemia	$R_C = R_N$	(12)
Adrenal carcinoma	$R_C > R_N$	(13)
Schizophrenia	$R_C = R_N$	(14)
Hypothyroid	$R_C > R_N$	(15)
Hyperthyroid	$R_C < R_N$	(15)
Endogenous depression	$R_C < R_N$	(16)
Chronic renal failure	$R_C > R_N$	(17)
Anorexia nervosa	$R_C < R_N$	(18,19)

[a] R = (THF + ATHF)/THE [tetrahydrocortisol + allotetrahydrocortisol)/tetrahydrocortisone]. R_C = subjects with designated condition. R_N = normal or control subjects.
[b] (1) Gray *et al.* (1962); (2) Bailey and West (1969); (3) Peterson and Pierce (1960); (4) Bush and Willoughby (1957); (5) Kornel (1970); (6) Zumoff *et al.* (1974); (7) Ichikawa (1966); (8) Pal (1967); (9) Zumoff *et al.* (1967); (10) Kornel *et al.* (1969); (11) Walker *et al.* (1991); (12) Gallagher *et al.* (1965); (13) Fukushima *et al.* (1960); (14) Romanoff *et al.* (1957); (15) Hellman *et al.* (1961); (16) Murphy (1991); (17) Walker and Edwards (1991); (18) Vierhapper *et al.* (1990); (19) Vanluchene *et al.* (1979).

shifts to the dominant postnatal ratio of 1–2 during the first year of life (Daniilescu-Goldinberg and Giroud, 1974; Savage *et al.*, 1975; Blunck, 1968; Kraan *et al.*, 1980); C. H. L. Shackleton, personal communication). The relationships between F and E in serum and amniotic fluid during the last trimester of pregnancy are similar to those of THF and THE (Noma *et al.*, 1991). So strong is the oxidation pressure in infants, that the blood *F/E* ratio will remain <1 even after intravenous administration of high concentrations of cortisol (maternal *F/E* = 11) (Buus *et al.*, 1966). The change in the 11-hydroxysteroid/11-oxosteroid ratio during early development is in accord with the changes in the increasing ability of 11-HSD to catalyze 11-reduction relative to 11-oxidation.

No data are available for the prenatal metabolism of corticosterone in humans. At the earliest known age examined, 1 year, the value for (THB + ATHB)/THA indicated a strong preference for the reduced

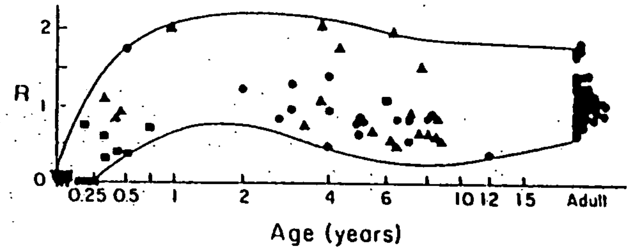


FIG. 3 The effect of age on the value of R = (THF + 5α THF)/THE. Modified from Monder and Shackleton (1984).

forms, THB and alloTHB (Peterson and Pierce, 1960; Savage *et al.*, 1975; Kornel *et al.*, 1969; Blunck, 1968). At every age, R_B exceeded R_F by two- to eightfold. The values for R_B fluctuated over a wide range between laboratories, and so from the limited data available, it is not possible to draw conclusions about age-related trends.

3. *C-11 Metabolism in Specific Organs*

Continuing the prenatal trend, 11-HSD increases in several organs during early postnatal development in the mouse (de Moor and Deckx, 1966) and rat (Hoff *et al.*, 1973), then decreases. In the mouse, 11-HSD, measured as oxidation of corticosterone at pH 10.5, rises from birth to 10 weeks of age in spleen, kidney, and liver, suggesting that it is due to some coordinated process, then declines to intermediate values. Development in the rat liver is qualitatively similar. Maximum value of cortisol oxidation occurs at 30 days of age, followed by a decline. Thus, in all species, alterations in the interconversion of corticosteroids at C-11 initiated in the fetus continue after birth, each organ following a unique pattern (Mitchell *et al.*, 1981; Pepe, 1979; Krozowski *et al.*, 1990; Moisin *et al.*, 1992).

IV. ARE 11-DEHYDROCORTICOSTEROIDS BIOLOGICALLY ACTIVE?

Cortisone is converted faster than cortisol to inactive metabolites: It binds poorly to glucocorticoid receptors (G. G. Rousseau *et al.*, 1972) under optimal experimental conditions and probably not at all under physiological conditions. On the basis of these observations it would not be predicted that 11-dehydrocorticosteroids have significant bio-

logical activity. There are, however, several observations that suggest otherwise. Barseghian *et al.* (1982) reported that cortisone, but not cortisol, strongly inhibited glucose- and arginine-induced insulin secretion. Berliner and Ruhmann (1966) found that though cortisol inhibited fibroblast growth in tissue culture, cortisone increased growth. In guinea pig isolated ileum, cortisone antagonized $GABA_A$ receptor-mediated contractile responses to applied GABA, in contrast with the enhancing effect of cortisol (Ong *et al.*, 1990). Souness and Morris (1990) have found that 11-dehydrocorticosterone caused significant kaluresis in male adrenalectomized rats and blocked the sodium ion retention caused by aldosterone. Souness and Morris (1991) have also found that, though 11-dehydrocorticosterone was not reduced to corticosterone by toad bladder preparations, the steroid nevertheless blocked aldosterone-stimulated short-circuit current.

There is one report, as yet unconfirmed, that 11-dehydrocorticosteroids have intracellular activity, mediating salt metabolism in the nasal gland of the domestic duck (Sandor *et al.*, 1983). The molecular basis for this observation is obscure. Membrane-associated events may explain the effects of cortisone on ileum contractile responses (Ong *et al.*, 1990); and possibly on other cortisone-mediated processes. The number of reports of 11-dehydrocorticosteroid activity are few, and none have been independently confirmed. Nevertheless, the examples cited support the possibility that oxidation of corticosteroid at C-11 may not be exclusively inactivating, and may generate physiologically significant metabolites.

V. 11-HSD in Lower Vertebrates

The presence of 11β-hydroxy- and 11-oxosteroids in animals as diverse as fish (Chan and Yeung, 1989; Gottfried, 1964), birds (Holmes *et al.*, 1974), and the platypus (McDonald *et al.*, 1988) indicates that 11-HSD serves an important function in nonmammalian vertebrates. Both cortisol and cortisone are found in salmon blood (Idler *et al.*, 1959a,b) and though oxidation may occur, cortisone does not appear to be effectively reduced to cortisol (Idler and Truscott, 1963). These conversions are probably extrahepatic, possibly occurring in the anterior kidney (Columbo and Bern, 1970), since in a wide range of bony fish there was no evidence of liver 11-HSD (Columbo *et al.*, 1972; Monder and Lakshmi, 1989a). However, generalizations about the role a particular organ in fish may play in 11-oxoreduction must be qualified. For example, in the ganoid fish, *Amia calva*, the anterior kidney was deficient in 11-HSD, unlike that of another ganoid, *Lepisosteus osseus* (Columbo *et al.*, 1972). The appearance in bile of cortisone and tetrahydrocortisone after injection of cortisol into trout (Truscott, 1979) or salmon (Donaldson and Fagerlund, 1972) is consistent with an active hepatic 11-HSD (Kime, 1978) in some species of teleost. The resolution of the source of 11-oxidoreduction in fish is of additional importance because C-11 steroids may be the source of the teleost androgens 11β-hydroxy- and 11-oxotestosterone (Idler and MacNab, 1967; Leitz and Reinboth, 1987; Rosenblum *et al.*, 1985). The somewhat more advanced African lung fish *Protopterus*, in contrast, is incapable of oxidizing the C-11 hydroxy group (Idler *et al.*, 1972). Early work on the occurrence of 11-oxygenated steroids in lower vertebrates have been summarized by Gottfried (1964).

Direct measurement of 11-HSD in livers of vertebrates has been made by Monder and Lakshmi (1989a). No 11β-dehydrogenase was detected in the liver microsomes of the frog, toad, mud puppy, shark, and several birds. In contrast, all mammals had activity. In the reductive direction, activity was present only in the livers of dogfish, birds, and mammals. Amphibians and teleosts had no detectable enzyme.

The duck nasal gland provides an interesting example of a system in which 11-oxidation may activate a steroid. Marine birds have a specialized organ, the nasal salt-gland, which protects them against the high salinity of ingested sea water. These glands concentrate and excrete the excess salt by a mechanism that is corticosteroid dependent. The endogenous corticosteroid, corticosterone, is rapidly oxidized to 11-dehydrocorticosterone by the nasal gland *in vivo*, *in vitro*, or by cell-free homogenates of the gland (Takemoto *et al.*, 1975; Sandor *et al.*, 1977). The glucocorticoid receptor, or an enzyme closely associated with the receptor, converts the specifically bound corticosterone to 11-dehydrocorticosterone (Sandor *et al.*, 1977, 1983; Sandor and Mehdi, 1980), which is transported to the nucleus. It is proposed that the receptor binds corticosterone, and its activation requires oxidation of the steroid at C-11.

VI. The Forms of 11-HSD Expression: Uniqueness or Multiplicity?

A. On the Question of Reversibility

The evidence presented thus far provides us with a picture of a functionally highly flexible enzyme, capable of adapting to net oxidation or net reduction depending on changing circumstances of age, health, state of gestation, and hormonal status. This remarkable

adaptive process engages the whole animal, and every organ within in unique and distinctive ways. The range of these processes is determined to some degree by genetic endowment (Nguyen-Trong-Tuan *et al.*, 1971). However, within these proscribed limits, the ability of the enzyme to respond to changing conditions is so striking that a closer look at it is justified. How is it possible for a single, presumably well-characterized enzyme to express itself as a net dehydrogenase under some conditions, and as a reductase under others?

A number of mechanisms have been proposed to account for the differential behavior of 11-HSD in various tissues and the changes in level of activity and in the directional characteristics of oxidoreduction that occur during development. Several rely on the properties of 11-HSD as a reversible pyridine nucleotide dependent oxidoreductase. Nicholas and Lugg (1982) and Torday *et al.* (1976) have postulated that the changing NADP/NADPH ratio is the driving force that determines the relative proportion of 11β-hydroxy- to 11-oxosteroid in lung in response to stress, castration, or adrenalectomy. Dougherty *et al.* (1960) utilized a similar mechanism to explain the appearance in immature lymphocytes of increased net 11-oxoreduction of cortisol subsequent to the introduction of triiodothyronine.

Other investigators have presented evidence that tissue specific changes in 11-HSD activities are not determined by the oxidation–reduction state of the tissue (Bernal *et al.*, 1980; Bernal and Turnbull, 1985) since they occur when nucleotide cofactors are not rate limiting. The effects of thyroxine in the rat persisted when pyridine nucleotide cofactors were not limiting (Zumoff *et al.*, 1983; Hellman *et al.*, 1961; Koerner and Hellman, 1964).

Product inhibition has been shown not to be responsible for the divergent effects of 11-HSD. Cortisol, even at 100-fold excess did not inhibit 11-oxoreductase activity (Bernal *et al.*, 1980). In the oxidative direction, neither NADPH nor 11-dehydrocorticosterone inhibited rat liver 11-HSD (Monder, 1991a).

An alternative hypothesis based on environmental perturbations is that the equilibrium ratio depends on pH. Changes in pH can theoretically affect the corticosteroid–11-dehydrocorticosteroid ratio, since the equilibrium of the overall redox reaction is dependent on the concentration of protons. The implementation of this hypothesis depends on knowledge of the equilibrium constant of the reaction and the local pH in the environment of the enzyme. Both are unknown. Changes of pH in the physiological range are not large enough to affect the 11-hydroxy/11-oxo ratio to a major degree (Lakshmi and Monder, 1985b; Monder and Shackleton, 1984). Any large local pH change that would persist for a sufficiently long time to alter the direction or magnitude of 11-HSD catalyzed reaction would adversely affect other processes in the endoplasmic reticulum.

Of the "environmental" hypotheses, the most likely mechanism accounting for small changes in oxidation–reduction properties is the one that proposes changes in the ratio of pyridine nucleotide cofactors. This may be an occasional mechanism for rapid, local perturbations in 11-oxoreduction. However, it can be readily shown that the ratios of reduced to oxidized pyridine nucleotides [NADPH/NADP] would have to be unrealistically high or low in order to account for the apparent extreme values of 11β-hydroxy/11-oxo in many tissues during development. Under physiological conditions, where the changes in 11-hydroxy/11-oxo in the whole organism based on urinary excretion patterns are not large, the relative proportion of reduced-to-oxidized cofactors may contribute to the behavior of the steroid at C-11. However, so many reactions occur in which both steroids and other biological substances require pyridine nucleotide cofactors within a cell, that is is not often that a circumstance arises where the machinery of the cell becomes entirely subservient to the metabolic requirements of a single molecule for any finite interval of time. Therefore, it is metabolically more likely and less disruptive for the cell to have developed other more specific mechanisms to control the interconversion of corticosteroids at C-11.

The possibility that the diverse behavior of 11-HSD in tissues is due to distinct, though related, enzymes has been considered by a number of investigators. In general, the view expressed has been that variants of 11-HSD are present in different tissues, representing forms with distinct kinetic properties that express behavior favoring reduction or oxidation. A model for this kind of system is glyceraldehyde phosphate dehydrogenase, in which different isozymes dominate in various tissues, and which have structural characteristics that lead to its preferential reduction to triose phosphate or oxidation to diphosphoglyceric acid (Kaplan, 1968). Thus the placental and decidual 11-HSD may be isozymes (Bernal *et al.*, 1980) as may also be true of the lung (Nicholas and Lugg, 1982) and liver (Bush and Mahesh, 1959b) enzymes.

B. Characteristics of Microsomal 11-HSD

1. *Latency*

The catalytic activity of the 11β-dehydrogenase component of 11-HSD is not fully expressed in liver microsomal preparations. Treatments that disrupt or alter the structure of the microsomal matrix,

such as phospholipase, detergent, and elevated pH, release latent enzyme activity. These processes, by altering membrane integrity, probably make the active site of 11β-dehydrogenase more accessible to its substrate (Gunderson and Nordlie, 1975). Latency of 11β-dehydrogenase, first observed in rat liver microsomes (Lakshmi and Monder, 1985b), occurs in the livers of other species, as well (Monder and Lakshmi, 1989a). This phenomenon is not unique. Other membrane-based enzymes express latency (Gunderson and Nordlie, 1975; Stetten and Burnett, 1967; Ernster and Jones, 1962; Schulze and Speth, 1980). It is possible that this property is a physiologically significant mechanism for controlling the expression of enzyme activity. In rat liver microsomal preparations, 11-oxoreductase activity is initially fully expressed without the intervention of latency releasing conditions (Lakshmi and Monder, 1985b). The latency behavior of hepatic 11β-dehydrogenase and 11-oxoreductase are therefore different.

2. *Energy of Activation*

The temperature dependence of enzyme activity can reveal much about the environment of the enzyme. The relationship of temperature and enzyme activity has been shown to adhere to thermodynamic principles and reflect the environment of the enzyme. The energy of activation is discrete in a homogeneous environment. If, however, the environment shows discontinuities, the energy of activation of an enzymatic process will show corresponding discontinuities should the activity be dependent on the structure of that environment (Raison *et al.*, 1971; Kumamoto *et al.*, 1971.

The energy of activation (E_a) of microsome-bound 11-dehydrogenase is continuous over the entire physiological temperature range and has the same value as the soluble enzyme. In contrast, microsomal 11-oxoreductase shows a discontinuity in E_a at 23°C, which is no longer present when the enzyme is solubilized, or when the microsomal lipid matrix is disrupted with phospholipases. The discontinuity coincides with a phase change in the matrix structure.

The differences between reductase and dehydrogenase with respect to latency and activation energy indicate that both activities are in distinct environments within the microsomal membrane. When they are solubilized, these environmental differences are removed, and the behavior of the enzymes reflect this (Lakshmi and Monder, 1985b).

3. *Enzyme Stability*

The relative stabilities of 11β-dehydrogenase and 11-oxoreductase provides an additional distinguishing criterion. Oxidation is the more

stable activity. With freshly prepared rat liver microsomal preparations, oxidation proceeds undiminished for at least 2 h at 37°C, whereas reductase is inactivated within 10 min.

We conclude from the literature and our own observations that there is sufficiently wide diversity in the properties and behavior of 11-HSD derived from different sources to be suggestive of multiple enzyme forms. The physicochemical and kinetic characteristics of the enzyme are also consistent with independent 11β-dehydrogenase and 11-oxoreductase sites. To address this question of enzyme multiplicity, it is necessary to review the molecular properties of 11-HSD. First, however, we will examine how the clinical evidence contributes to our understanding of its properties and physiological functions.

VII. Clinical Studies

A. 11β-Dehydrogenase Deficiency

1. *Apparent Mineralocorticoid Excess*

In humans, an "experiment of nature" has provided insight into the probable function of 11-HSD in at least one organ, the kidney. A disease apparently unique to children was described in the 1970s with a clinical picture consisting of low renin activity, low aldosterone production, hypokalemia, and severe hypertension (Ulick *et al.*, 1977; Werder *et al.*, 1975; Ramirez *et al.*, 1979; Winter and McKenzie, 1977). The first completely described patient with this condition was a Zuni Indian girl in whom the diagnosis was made at the age of 3 years (New and Levine, 1977; New *et al.*, 1977). Urinary cortisol and deoxycorticosterone metabolites were below normal and were not increased after ACTH stimulation. Glucocorticoid administration exacerbated the hypertension, suggesting that it was in some manner linked to endogonous cortisol.

Extreme sodium deprivation lowered blood pressure, possibly by stimulating the rate of conversion of corticosterone to aldosterone (Haning *et al.*, 1970). High doses of spironolactone, a potassium-sparing diuretic that acts via blockade of the mineralocorticoid receptor, also produced normalization of blood pressure, and on this regimen, plasma renin activity rose. Substitution of triamterene, a potassium-sparing diuretic that does not affect the mineralocorticoid receptor, failed to ameliorate blood pressure.

In vivo measurement of transcolonic electrical potential difference

in the patient was consistent with mineralocorticoid effect seen in patients with primary hyperaldosteronism; the potential difference was increased with hydrocortisone administration, and diminished with spironolactone administration (New *et al.*, 1982). Sensitivity of glucocorticoid receptors was normal as assayed in lymphocytes (Bigger *et al.*, 1972). Bioassays performed to demonstrate the presence of a steroid hormone in the patient's serum capable of causing sodium retention revealed no mineralocorticoid effect (Marver and Edelman, 1978; Blair-West *et al.*, 1962; Sennett *et al.*, 1975; Adam *et al.*, 1978; Baxter *et al.*, 1976). Because the symptoms and response to treatment were consistent with aldosteronism, despite the low circulating levels of the steroid, the syndrome was referred to as "apparent mineralocorticoid excess" (AME). This designation appears to have gained general acceptance.

Patients with AME have shown (*a*) low rate of cortisol turnover, with approximately twice the disappearance time of radiolabeled tracer steroid compared with that of a normal subject; (*b*) low peripheral plasma ACTH levels; (*c*) normal CBG concentration; (*d*) greatly diminished level of urinary metabolites of cortisone compared with those of cortisol; (*e*) no production of tritiated water after infusion of 11α-[^{3}H]cortisol, suggesting a defect in the oxidative component of the 11β-hydroxysteroid dehydrogenase (Ulick *et al.*, 1979); (*f*) normal metabolism of cortisone to cortisol, suggesting an intact reductive component of 11β-hydroxysteroid dehydrogenase (Ulick *et al.*, 1979; Monder *et al.*, 1986); and (*g*) an abnormal increase in the 5α- relative to 5β-metabolites of cortisol (Ulick *et al.*, 1977).

Selective glucocorticoid receptor (GR) blockade with RU 38486 (RU 486) did not decrease blood pressure as would be expected if the GR were responsible for the development of hypertension in AME. Instead, a significant increase in mean blood pressure was observed compared with the pretreatment period, indicating that the GR was not contributing to the development of hypertension.

The constellation of clinical, hormonal, and metabolic features that have been described in patients with AME including sib pairs (DiMartino-Nardi *et al.*, 1987; Shackleton *et al.*, 1985) suggests an inborn error of metabolism attributable to a defect in the gene encoding 11β-hydroxysteroid dehydrogenase (New *et al.*, 1982; Oberfield *et al.*, 1983). Attempts to evince 11-HSD deficiency in parents have yielded positive results in one father whose excretion of tritiated water was slightly low compared with controls (M. I. New, P. Speiser, and H. L. Bradlow, unpublished) and in one mother with mild hypokalemia and hypertension (Stewart *et al.*, 1988). The fact that a subtle enzyme defect could not consistently be demonstrated in parents of these patients (Shackleton *et al.*, 1985; DiMartino-Nardi *et al.*, 1987) does not negate the genetic theory.

Apparent mineralocorticoid excess occurs in all racial groups and is equally distributed between males and females (Stewart *et al.*, 1987) (Table VIII). Among patients identified to date, ages at diagnosis have ranged from 5 months to 20 years. The fact that no adults with the condition have been described suggests that the disease, if untreated, is invariably fatal. Five patients have died, yielding a mortality rate of 25%. Most patients had some evidence of end organ damage at the time of diagnosis. Two patients had severe complications of aortic insufficiency, one requiring aortic valve replacement. Although the initial therapeutic response to mineralocorticoid blockade with spironolactone is good, patients eventually require two to three antihypertensive medications to maintain their blood pressure within a safe range. It is not well understood why the hypertension in this syndrome follows a more malignant course than in other forms of mineralocorticoid-induced hypertension.

2. *Licorice Ingestion*

Studies of licorice ingestion provide further insight into the mechanism of glucocorticoid-mediated hypertension. A decade after the first complete description of a patient with AME, Stewart, Edwards, and colleagues were able to show that when healthy adult males were given 200 g/day of licorice (containing 580 mg glycyrrhizic acid, the active component of the confection) their hormonal and metabolic profiles paralleled the profile of AME patients (Stewart *et al.*, 1987). This led to the crystallization of a proposal first promulgated by New in 1982 (New *et al.*, 1982): An increase in cortisol versus metabolically inactive cortisone causes saturation of cortisol binding globulin, allowing cortisol to gain access to the mineralocorticoid receptor (MR), which shows no intrinsic preference for aldosterone as a ligand. Thus, the 11-HSD is the integral link in protecting renal MR from the normally extant 1000-fold excess physiologic concentration of cortisol compared with aldosterone (Edwards *et al.*, 1988; Funder *et al.*, 1988).

Recent evidence suggests that the metabolic effects of carbenoxolone and glycyrrhetinic acid, in contrast with their clinical effects, may differ from each other. Stewart and Edwards (1991) have shown that carbenoxolone, in contrast with glycyrrhetinic acid, did not change urinary (alloTHF + THF)/THE, or alter plasma cortisone in volunteers. The metabolic profile resembles that of a form of AME reported by Ulick.

TABLE VIII

REPORTED CASES OF APPARENT MINERALOCORTICOID EXCESS (AME)

Patient[a]	Patient age[b] (years)	Sex	R[c]	Blood pressure (mm Hg)	Aldosterone[d] (ng/dl)	Citation[e]
1	3	F	10.2	175/115	1.9	(1)
2D (14 years)	3	F	>7	144/104	ND	(2)
3D (12 years)	0 1/12 (2 9/12)	F	9.8	180/120	ND	(3)
4	1 7/12	M	>4	140/100	ND	(3)
5	9	M	>10	250/180	ND	(4)
6	3 3/12	F	40	125/85	—	G. Phillipou (1978)[f]
7	2 (4)	M	15.9	140/90	1.3	(5)
8D	1 7/12	M	45	150/110	3	(6)
9D (5/12 year)	0 5/12	M	70	200/100	1.1	(7)
10	0 9/12 (19)	F	15	150/100	2.4	(8)
11R	3	F	31.2	170/110	<0.2	(9)
12R	3 9/12	F	13.4	200/129	—	(9)
13	7	F	29.8	160/120	ND	(10)
14	9	M	26.9	170/100	—	J. S. D. Winter (1988)[f]
15	3	M	7.5	200/110	—	Peskovitz (1986)[f]
16	21	M	13.5	200/145	<3.4	(11)
17R	2 (9 4/12)	F	8.9	130/90	ND	(12)
18R	2 6/12 (4 4/12)	F	20	142/98	ND	(12)
19	14 9/12	M	8	130/90	ND	(12)
20	2 3/13	M	129			Wood (1992)[f]

[a] D, patient died (age at death); R, the adjacent patients are siblings.

[b] Two ages are presented in some cases. The first is the one in which hypertension was reported. The second, in parenthesis, is the age at which AME was diagnosed. A single figure indicates that hypertension was found at the time of AME diagnosis.

[c] $R = (\mathrm{THF} + 5\alpha\mathrm{THF})/\mathrm{THE}$.

[d] Normal range is 5 to 20 ng/dl. ND, not detected.

[e] (1) Werder *et al.* (1975); (2) New *et al.* (1977); (3) Winter and McKenzie (1977); (4) Ulick *et al.* (1979); (5) Shackleton *et al.* (1980); (6) Fiselier *et al.* (1982); (7) Honour *et al.* (1983); (8) Harinck *et al.* (1984); (9) Shackleton *et al.* (1985); (10) Batista *et al.* (1986); (11) Stewart *et al.* (1988); (12) Monder *et al.* (1986).

[f] Unpublished.

3. *Alternative Forms of AME*

Ulick has described a Type 2 AME in which the cortisol metabolic clearance rate is delayed, but the conversion of cortisol to cortisone is not impaired (Ulick *et al.*, 1989). Unlike patients with the classic form of AME, hypertension in the Type 2 patients is ameliorated by treatment with dexamethasone (Ulick *et al.*, 1990). It has been suggested that these cases might be explained based on a generalized defect in cortisol metabolism. Alternatively, the absence of a discernible alteration in the THE:THF ratio may reflect equivalent defects in both the oxidative and the reductive components of the 11-HSD system. Support for the latter theory derives from *in vivo* studies with carbenoxolone in which cortisol half-life was prolonged, yet the THE:THF ratio was not perturbed (Stewart *et al.*, 1988).

Liddle has described a familial hypertensive syndrome with signs of mineralocorticoid excess responsive to triamterene, but not to spironolactone (Liddle *et al.*, 1963). The proposed etiology for this disorder is enhanced renal tubular sensitivity to low levels of mineralocorticoids; several additional cases have since been reported (Milora *et al.*, 1967; Helbock and Reynolds, 1970; Wachtel *et al.*, 1975; Costin *et al.*, 1979; Wang *et al.*, 1981).

Although the syndrome of AME usually results in severe and often fatal hypertension and has most often been diagnosed in children (New and Levine, 1977; New *et al.*, 1977; Winter and McKenzie, 1977; Werder *et al.*, 1974; Fiselier *et al.*, 1982; Honour *et al.*, 1983; Harinck *et al.*, 1984; Shackleton *et al.*, 1985), one adult-onset case has been recognized (Stewart *et al.*, 1988). This patient was thought to have the classic form of AME, but unlike most of the others reported, he was responsive to dexamethasone treatment in terms of restoring positive potassium balance and elevation of plasma renin activity, although blood pressure was not significantly changed. Other classic cases where dexamethasone was tried with some salutary efects were reported by Werder *et al.* (1974) and others (Fiselier *et al.*, 1982; Honour *et al.*, 1983; Harinck *et al.*, 1984; Shackleton *et al.*, 1980). Secondary effects of apparent mineralocorticoid excess have also been reported, most notably the coexistence of large renal calculi (DiMartino-Nardi *et al.*, 1987), and in one case actual rickets due to secondary hyperparathyroidism (Batista *et al.*, 1986).

Clinical characterization of the syndrome of apparent mineralocorticoid excess has provided unique and powerful insights into the importance of 11β-hydroxysteroid dehydrogenase in blood pressure homeostasis. For the clinician, there are as yet several unresolved questions: (1) Why are these patients not Cushingoid in light of the low

plasma ACTH and accompanying prolonged cortisol half-life? (2) Conversely, if they are not in a state of cortisol excess as reflected by low plasma ACTH levels, how are they able to survive stressful illness without cortisol supplementation? (3) What are the relationships between the variant syndromes that have been described? (4) Why is there so much heterogeneity among patients with respect to the therapeutic efficacy of low-sodium diet, spironolactone, triamterene, and dexamethasone?

4. *The Defect in AME is Mainly in the Kidney*

That the primary defect of AME was in the kidney tubule was deduced from the fact that hypertension and salt imbalance was controlled using the therapeutic regimen utilized for the treatment of aldosteronemia, despite clear evidence for hypoaldosteronemia in these patients. How the imbalance in conversion of cortisol to cortisone is related to juvenile hypertension emerges from the observation that, in all species, cortisol (and corticosterone) at sufficiently high concentrations are mineralocorticoids. Under normal physiological conditions, the active mineralocorticoid is aldosterone; glucocorticoids have no important role in salt metabolism. It is now known that MR bind aldosterone and corticosterone (or cortisol) with equally high affinity (Amelung *et al.*, 1953b). Funder (1987) posed the following significant question: how does aldosterone get its message through to the mineralocorticoid target tissues in the face of much higher circulating free levels of the glucocorticoids? In attempting to answer this question, Stewart and Edwards (1990) and Funder (1990a) have presented a refined and expanded version of earlier proposals (New *et al.*, 1982) that were designed to explain the role of 11β-dehydrogenase in blood pressure control. They proposed that the role of 11β-dehydrogenase in highly vascular tissues, such as the kidney, is to provide an enzymatic barrier to prevent the accumulation of levels of glucocorticoid that would compete with aldosterone for MR. In AME, this barrier is defective, aldosterone secretion is suppressed, and cortisol, because it cannot be inactivated, is utilized by the receptor as if it were a mineralocorticoid.

B. 11-Oxoreductase Deficiency

Independent reports by Taylor *et al.* (1984) in England and Phillipou (Phillipou and Higgins, 1985) in Australia described female patients with apparent deficiency of 11-reduction. These women presented with hirsutism and bilaterally enlarged adrenal glands. Plasma androgen concentrations were about five times above normal; plasma and urinary free cortisol were normal. Examination of the urinary steroids revealed a 7- to 9-fold increase in cortisol metabolites and a 6- to 10-fold increase in androgens. The ratio of THE/(THF + 5αTHF) was extremely high (26, normal ca. 1). These are the only recorded examples of selective 11-oxoreductase deficiency. The evidence indicates two conditions, AME and 11-oxoreductase deficiency, in which 11-HSD appears to be expressed in opposite directions with little reversibility.

VIII. Enzymology and Molecular Biology

A. The Uniqueness of 11-HSD

The metabolism of a steroid in its target cell determines its effective intracellular concentration, its accessibility to its receptor, and its ability to affect cell function. Thus, any catabolizing enzyme could qualify as a candidate for controlling tissue steroid levels. In this sense, the role of 11-HSD is potentially not different from that of any other enzyme. There are, however, characteristic properties of 11-HSD that, taken together, make it unique. First, 11-HSD affects the activity of glucocorticoids and no other steroid class. Second, 11-HSD is the dominant, if not the sole, enzyme responsible for modifying intracellular corticosteroid levels in many tissues. Third, the enzyme is reversible, enabling it to control the direction of corticosteroid metabolism, thus permitting it to catalyze 11-oxidation to diminish intracellular glucocorticoid concentrations, or 11-reduction to increase them. Fourth, in circumstances where selectivity of aldosterone activity is crucial, such as in the kidney or brain, the enzyme specifically depletes glucocorticoid, without affecting mineralocorticoid.

B. Preparation and Properties of Homogeneous 11-HSD

1. *Purification*

The selective directionality of 11-HSD catalysis has led to numerous hypotheses, some assuming a unique reversible enzyme, others a complex of separate, intercommunicating proteins expressing either 11β-dehydrogenase or 11-oxoreductase activities. Attempts to separate these activities or purify 11-HSD have, in the past, been unsuccessful (Hurlock and Talalay, 1959; Bush *et al.*, 1968). The enzyme of rat liver is embedded in the endoplasmic reticulum, and because of this, its

purification presents particular problems unique to membrane-bound proteins. Release of the protein from the membrane without denaturing it is usually achieved by displacing the detergent-like native environment with a synthetic detergent (Hjelmeland and Chrambach, 1984; Helenius and Simons, 1975; Tanford and Reynolds, 1976; Rajin, 1972; Lakshmi and Monder, 1985a). Detergent extraction releases 11-HSD in a soluble state, but does not separate oxidation and reduction activity (Lakshmi and Monder, 1986a).

To investigate the properties of 11-HSD, it was purified from rat liver using NADP–agarose affinity chromatography. The homogeneous enzyme preferentially used NADP as cosubstrate; NAD was about 30% as effective (A. Marandici and C. Monder, unpublished observations). The enzyme expressed no detectable 11-oxoreductase activity. This observation initially reinforced the conclusion that 11-HSD is a complex of separate 11β-dehydrogenase and 11-oxoreductase components (Lakshmi and Monder, 1988).

2. *Properties of Purified Enzyme*

The homogeneous 11β-dehydrogenase is a glycoprotein with a monomer molecular weight of about 34,000. It readily aggregates into clusters of 5 to 11 units, due to the mutual attraction of its hydrophobic regions. Total liver 11-HSD activity is the sum of high K_m (6 μM, corticosterone as substrate) and low K_m (90 nM) activities. Purified enzyme expresses the kinetic behavior of the high K_m form (Monder and Lakshmi, 1989b).

Kinetic analysis and ligand binding studies of purified 11-HSD reveals that the behavior of the enzyme conforms to an ordered sequential mechanism (Monder *et al.*, 1991). In the oxidative direction, the obligatory sequence of addition of cosubstrates requires that NADP be bound first, followed by corticosteroid. Because the enzyme does not express 11-oxoreductase activity, no kinetic analysis has been possible in the reductive direction.

3. *Antibodies*

Monospecific, polyclonal antibodies to homogeneous rat liver 11-HSD generated in rabbits (Monder and Lakshmi, 1990) have been used to investigate the organ-specific distribution and physiological functions of this enzyme in several organs (Monder, 1991a,b). In all tissues of the rat thus far investigated, 11-HSD antibody reveals a 34K protein indistinguishable from that of the rat liver enzyme (Monder and Lakshmi, 1990). The intensities of the bands on electrophoretograms after Western blot analysis generally corresponded in magnitude with enzyme activity. A few tissues that expressed 11-HSD activity had no evidence of 11-HSD-like immunoreactivity, suggesting that they contain possible alternative enzyme forms (Monder, 1991a).

C. Molecular Analysis

1. *Structure–Function Predictions*

As a first step in the molecular genetic analysis of this enzyme, clones encoding 11-HSD were isolated by probing a rat liver cDNA library in the phage λgt11 with a monospecific antiserum to 11-HSD (Agarwal *et al.*, 1989). Analysis of clones demonstrated that the mRNA encoding this enzyme in the rat has an open reading frame that predicts a polypeptide of 287 residues with a molecular weight of 31,800, in contrast to the purified protein's actual MW of 34,000. The difference may be due to glycosylation; there were two potential sites for N-glycosylation in the predicted sequence. The rat clone was subsequently used to isolate human 11-HSD cDNA clones from a testis library (Tannin *et al.*, 1991). The amino acid sequence of human 11-HSD predicted from the nucleotide sequence is 79% identical to the corresponding rat sequence.

A search of sequence databases revealed that the predicted sequence of 11-HSD was related to several other prokaryotic and eukaryotic enzymes (Baker, 1989, 1990a). These include steroid 3α,20β-hydroxysteroid dehydrogenase from *Streptomyces hydrogenans* (Marekov *et al.*, 1990), a murine 27-kDa adipocyte protein of unknown function, the *nodG* gene product of the nitrogen fixing bacterium *Rhizobium meliloti*, ribitol dehydrogenase from *Klebsiella aerogenes*, the *act III* gene product from *Streptomyces coelicolor*, human estradiol 17β-hydroxysteroid dehydrogenase, and dihydrodiol dehydrogenase from *Pseudomonas* species. Although it could not be readily aligned directly with 11-HSD, alcohol dehydrogenase of *Drosophila melanogaster* showed significant similarity to several of the other dehydrogenases used in the alignment (Baker, 1990b). Examination of these alignments (excluding *Drosophila* alcohol dehydrogenase) revealed a total of nine residues that were conserved in all proteins. These residues are likely to be structurally or functionally important. Although it, too, could not be aligned with 11-HSD using the computer algorithm, human 3β-hydroxysteroid dehydrogenase retains six of these nine residues in a similar arrangement (Fig. 4). Three of these residues are in an area near the amino terminus that is similar to known nucleotide cofactor binding sites of other enzymes, including

yeast alcohol dehydrogenase (Jornvall *et al.*, 1981). If the three absolutely conserved residues distal to the cofactor binding site (Asp-114, Tyr-183 and Lys-187, human 11-HSD; Asp 110, Tyr-179, Lys-183, rat liver 11-HSD) participate in the catalytic function of the enzyme, they should be near the pyridine ring of NADP$^+$ and/or the 11α position of the steroid, a hypothesis that could be tested if the three-dimensional structure of 11-HSD could be determined by X-ray crystallography.

The three-dimensional structure of a related enzyme should also provide useful information concerning the functional significance of the conserved residues. Crystallographic studies of 3α,20β-hydroxysteroid dehydrogenase of *S. hydrogenans* were recently reported (Ghosh *et al.*, 1991),. In this related enzyme, the conserved region near the amino terminus does form part of the nucleotide cofactor binding site. The conserved tyrosine residue (Tyr-152 in 3α,20β-HSD) is indeed located near the pyridine ring of the cofactor in a cleft that is presumed to be the steroid binding site. The conserved lysine is directly behind the tyrosine (i.e., on the opposite side of the tyrosine ring from the cofactor). There is demonstrable bridging of electrons between the phenolic hydroxyl of tyrosine and the δ-amino group of lysine, suggesting an interaction between these groups. These findings support the idea that the conserved tyrosine and lysine participate in the catalytic function of the enzyme by facilitating the transfer of a hydride radical from the steroid to the cofactor. In contrast, the conserved aspartate (Asp-82 in 3α,20β-HSD) is not located near the cofactor or the steroid, and its functional significance is difficult to assess from these studies. Thus, it will be necessary to test the importance of these residues in 11-HSD by *in vitro* mutagenesis and expression of the cDNA in cultured cells.

2. *Functional Characteristics of Recombinant 11-HSD*

To determine whether both 11β-dehydrogenase and 11-oxoreductase activities resided in the same enzyme, a full-length cDNA clone was expressed in Chinese hamster ovary (CHO) cells by transient transfection with a plasmid expression vector. Enzymatic activities were determined by incubating transfected cells with radioactive substrates. Whereas normal CHO cells did not contain significant 11β-dehydro-

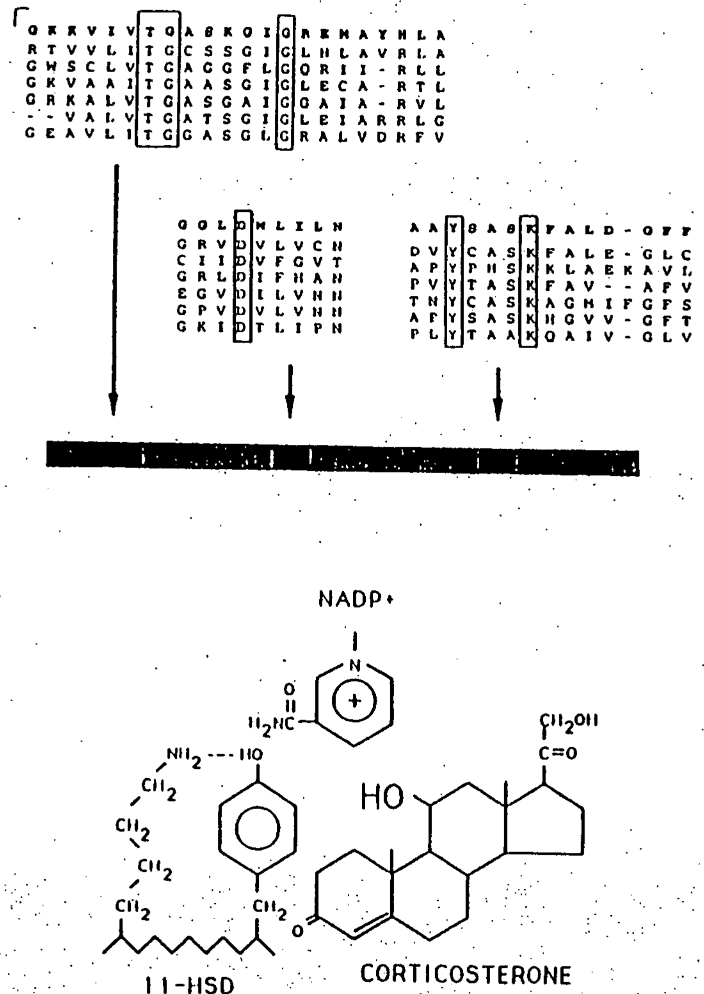


FIG. 4. (Top) Conserved amino acid sequences in 11β-hydroxysteroid dehydrogenase (bold letters) and related enzymes. In descending order, the sequences are 11-HSD, 17β-hydroxysteroid dehydrogenase, 3β-hydroxysteroid dehydrogenase, ribitol dehydrogenase (Klebsiella aerogenes), actIII protein from *Streptomyces coelicolor*, dihydrodiol dehydrogenase from *Pseudomonas* sp. Amino acids shown are A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. Absolutely conserved residues are boxed. The positions of these sequences are indicated by the dark boxes within the shaded box depicting the 11-HSD amino acid sequence. (Bottom) Proposed active site of rat liver 11-HSD showing the spatial relationships of tyrosine-179, the pyridine ring of NADP$^+$, lysine-183, and position 11 of the steroid substrate.

genase and 11-oxoreductase activities, these cells developed roughly equal levels of both activities (about 40% conversion of substrate to product after 20 h) after transfection with the expression plasmid. Addition of glycyrrhetinic acid, a known inhibitor of 11β-dehydrogenase, reduced expressed dehydrogenase activity by 50% without affecting reductase activity (Lakshmi and Monder, 1985b).

To obtain kinetic parameters for the two activities, 11-HSD was expressed at higher levels using recombinant vaccinia virus (Agarwal *et al.*, 1990). Dehydrogenase and reductase activities were assessed in cellular lysates in the presence of saturating concentrations of NADP and NADPH, respectively. At pH 7.0, the recombinant enzyme had very similar K_m and first-order rate constants (V_{max}/K_m) for both activities. These results were consistent with the hypothesis that both dehydrogenase and reductase activities reside in a single enzyme. Exposure to NADP resulted in rapid and irreversible inactivation of the reductase activity of the enzyme, a phenomenon consistent with the instability of the reductase during attempted purification from rat liver.

In contrast, when the recombinant enzyme was prepared from cells grown in the presence of A_1 tunicamycin (an inhibitor of glycosylation), dehydrogenase activity was reduced by about 50%, whereas reductase activity was unaffected. This was associated with increased amounts of a 31-kDa enzyme species that presumably represented the unglycosylated enzyme. This suggests that the dehydrogenase activity of the enzyme may depend on adequate glycosylation.

3. *Tissue Distribution of 11-HSD Expression*

In initial studies, the rat cDNA clone hybridized to a single mRNA species of approximately 1600–1700 nucleotides in samples from testis (highest), liver, kidney, and lung but did not hybridize to samples from heart or colon. This distribution roughly paralleled that of 11β-dehydrogenase activity.

A subsequent study (Krozowski *et al.*, 1990) suggested that the rat kidney actually contains several cross-hybridizing mRNA species of 1900, 1600, and 1500 nucleotides (renal cortex/medulla) and 1700 nucleotides (renal papilla). In this study, the highest level of expression was found in the liver, followed respectively by kidney, lung, testis, hippocampus, heart, and colon.

In further studies of expression in rat brain (Moisin *et al.*, 1990a,b), an apparently identical mRNA species was found in all areas, but at highest levels in the hippocampus and cortex. It is speculated that 11-HSD regulates the access of glucocorticoids to cerebral mineralocorticoid and/or glucocorticoid receptors, thus modulating steroid hormone effects of cerebral function.

The tissue distribution of the human mRNA differs from that in the rat; it is expressed at very high levels in the liver and at much lower levels in the kidney. The significance of these findings, given the importance of this enzyme activity in the kidney, is not yet clear, but it is consistent with the idea that there may be additional proteins with 11-HSD activity in the kidney.

4. *Genetic Analysis of Human 11-HSD*

To determine the chromosomal location of the human 11β-hydroxysteroid dehydrogenase (*HSDB11*) gene, a cDNA clone was hybridized to DNA samples from a panel of human–rodent somatic cell hybrid lines. Hybridization to human-specific bands was consistent with a location on chromosome 1 (Tannin *et al.*, 1991).

Hybridization of blots of uncloned human genomic DNA that had been digested with restriction endonuclease *Hin*dIII demonstrated that there was a single *HSD11* gene that was carried on two fragments. Sequence analysis of these fragments showed that they carried a single gene consisting of six exons, the first four of which were contained on the smaller fragment. Comparison of the maps of restriction sites in these fragments with results of hybridization to uncloned DNA revealed that there must be an additional *Hin*dIII fragment(s) of undetermined size in intron 4 that contains *Eco*RI and *Bam*HI sites.

a. Transcriptional Regulation of the HSD11 Gene. Primer extension analysis indicated that transcription of the human *HSD11* gene starts 93 bp upstream from the start of translation (Tannin *et al.*, 1991). This yields a 5′ untranslated region very similar in length to that of rat 11-HSD mRNA. There is no TATA box in the 5′ flanking region, but there is a consensus CAAT box (CCAATC) 76 bases upstream from the start of transcription. An 8-bp palindromic sequence (CTGTACAG) was present 188 bp upstream from the start of transcription. It resembles part of a glucocorticoid response element (Evans, 1988), which would be consistent with the known ability of glucocorticoids to increase levels of 11-HSD activity. However, its functional significance requires further study, particularly in light of recent work suggesting that glucocorticoids do not alter the level of *HSD11* gene expression in rat liver, lung, or kidney (Krozowski *et al.*, 1990).

Recent S1 nuclease analysis suggests that the different-sized mRNA transcripts observed in rat kidney apparently have different 5′ extensions. Cloning studies suggested that some transcripts have a divergent 5′ coding sequence that encodes a putative protein with a

truncated amino terminus (Krozowski *et al.*, 1992). Comparison of the sequence of the truncated clones with that of the human gene suggests that these clones originate by transcription within the first intron of the corresponding rat gene. It is not yet known whether the putative protein is functional or even whether it is synthesized *in vivo*.

b. Possibility of Additional 11-HSD Enzymes. In addition to the putative truncated form of the protein, other evidence suggests that there may be an additional enzyme(s) with 11-HSD activity. As mentioned, there appears to be some discrepancy between the levels of 11-HSD activity and *HSD11* mRNA in human kidney. In rat kidney, anti-11-HSD sera react with proximal tubules but not with distal tubules/collecting ducts, although the latter represent the main site of mineralocorticoid action (Rundle *et al.*, 1989a). The 11-HSD activity of isolated rabbit distal tubules and collecting ducts differs markedly in kinetic properties from the enzyme in the proximal tubule (which is similar or identical to the enzyme in the liver) in having a K_m about 100-fold lower (Naray-Fejes-Toth *et al.*, 1990). A similar low K_m form has been detected as a minor species in the liver (Monder and Lakshmi, 1989b). Furthermore, histochemical studies have suggested that the distal tubule contains an 11-HSD activity that requires NAD rather than NADP (Mercer and Krozowski, 1992). No mRNA was detected in heart, yet heart readily converts cortisol to cortisone (C. Monder and A. Marandici, unpublished observations) (Kolanowski *et al.*, 1981). Because Southern blotting studies indicate that rats and humans carry only one *HSD11* gene, the gene(s) encoding any additional 11-HSD activities must be sufficiently different from *HSD11* in their nucleotide sequences that they do not cross-hybridize.

A number of questions regarding the functions of 11-HSD may be answered by molecular genetic analysis of patients with inherited enzymatic deficiencies. Because both dehydrogenase and reductase activities apparently reside in the same enzyme, it will be of obvious interest to search for mutations in the HSD11 gene(s) associated with AME and 11-oxoreductase deficiencies and correlate their effects on enzymatic function with clinical phenotype.

IX. 11-HSD Function in Specific Organs

A. Kidney

1. *Mineralocorticoid Receptors and 11-HSD*

We have discussed the fact that the characteristic biochemical abnormality of AME is a severe loss in the ability of patients with this

disability to oxidize cortisol. A working model connecting 11-HSD activity and blood pressure control evolved from the convergent findings of many laboratories. It was discovered that, *in vitro*, the renal MR bound aldosterone and the glucocorticoids, corticosterone and cortisol, with comparable affinity (Krozowski and Funder, 1983; Arriza *et al.*, 1987; Armanini *et al.*, 1985). That this behavior is an intrinsic property of MR was shown by Arriza *et al.* (1987) using cloned recombinant MR derived from placental cDNA expressed in COS cells. However, *in vivo*, aldosterone was selectively taken up by the MR of some tissues, such as kidney, parotid, and colon (Sheppard and Funder, 1987a,b), to the exclusion of glucocorticoids. That these tissues are aldosterone selective appeared to be in conflict with the *in vitro* data. To reconcile the *in vitro* and *in vivo* evidence, a hypothesis to explain steroid selectivity was developed based on the observation that glucocorticoids are uniquely sequestered to corticosteroid binding globulin (CBG) and are thus made unavailable to MR (Krozowski and Funder, 1983; Sheppard and Funder, 1987a). It was, however, found that aldosterone selectivity persisted *in vivo* in young rats with little or no circulating CBG (Sheppard and Funder, 1987a,b). Since MR is coded for by a single gene (Arriza *et al.*, 1987), it was considered unlikely that selectivity depends on tissue- or age-specific variations in its intrinsic properties. However, hormone-dependent gene regulation by MR showed a preference of aldosterone over cortisol (Arriza, 1991). It was recently suggested that post-translational modifications of MR may play a role in mineralocorticoid binding and specificity, but this possibility remains to be evaluated (Doyle *et al.*, 1988). A search for specific regions of MR localization in the rat nephron revealed that MR are not distributed uniformly throughout the renal tubule and are localized to the distal tubule. Krozowski *et al.* (1989), using an antiserum corresponding to the hinge region of human MR (Arriza *et al.*, 1987), showed that MR are localized in the principal cells of the cortical collecting duct. MR were not detected in the proximal tubule and glomerulus (Wrange and Yu, 1983). They have been reported to be present in the distal convoluted tubule and the thick ascending limb of the loop of Henle (Doucet and Katz, 1981; Katz, 1990; Farman *et al.*, 1983). Thus, although there is some uncertainty about the range of MR distribution in the distal tubule, its localization to this region is not questioned (Krozowski *et al.*, 1989; Rundle *et al.*, 1989b; Bonvalet, 1991).

The renal distribution of immunoreactive 11-HSD and MR was very different. Polyclonal antibodies (Edwards *et al.*, 1988; Rundle *et al.*, 1989a) and monoclonal antibodies (Castello *et al.*, 1989) to 11-HSD revealed specific immunoreactive staining in the proximal tubules of the inner cortex. The focal distribution of 11-HSD and MR within the

tubule suggested a model in which blood filtrate containing aldosterone and a thousandfold greater concentration of corticosterone must pass through a region of high 11-DH activity, located in the proximal tubule and vasa recta. During this passage corticosterone is completely inactivated, leaving the aldosterone, which is not a substrate of the enzyme, unaltered, permitting its binding to MR to occur unopposed. Since 11-DH and MR do not colocalize, a paracrine relationship between them is inferred. An explanation of the consequences of the deficiency of 11-DH in humans is predicated on the assumption that these structural and functional relationships apply to human as well as to rat kidney.

2. *Role of Glucocorticoid Receptors*

The hypothesis based on this model assumes that all available glucocorticoid is oxidatively inactivated as it passes through the region of high 11-HSD activity. The model in this form proved to be extremely useful and enabled puzzling aspects of AME to be explained. It is, however, known that the renal tubule contains glucocorticoid receptors. These mediate glucocorticoid-specific effects on the kidney including effects on renal hemodynamics, acid and water excretion, gluconeogenesis, and sodium–potassium ATPase (Katz, 1990; Kinsella, 1990). Consequently, complete inactivation of corticosterone or cortisol would not be a desirable option for the kidney. Therefore, the hypothesis originally proposed that envisioned a relationship between the MR and 11-HSD that excludes GR-mediated effects must be revised. Glucocorticoid receptors are known to be distributed along the nephron (Farman *et al.*, 1991; Katz, 1990). The number of glucocorticoid binding sites in the thick ascending limb of the loop of Henle, for example, is 100-fold higher than the aldosterone binding sites (Lee *et al.*, 1983). Maximal binding capacity of the cortical collecting tubule for corticosterone is greater than for aldosterone (Katz, 1990). Thus, though glucocorticoids at moderate concentrations may mediate renal function by way of MR, GR are important, as well (Naray-Fejes-Toth and Fejes-Toth, 1990; Clore *et al.*, 1988). The emerging concept that 11-HSD plays an important role in mediating GR dependent processes is supported by the observation that there is a strong correlation between GR and 11-HSD distribution in tissues (Whorwood *et al.*, 1992).

3. *The Protector Role of 11-HSD: Modifying the Hypothesis*

The hypothesis that renal 11-HSD enables MR to interact selectively with aldosterone as both ligand and effector by inactivating potentially competing glucocorticoids was supported by extensive laboratory and clinical data (Funder, 1990a,b; Stewart and Edwards, 1990; Edwards *et al.*, 1989; Monder and Shackleton, 1984; Monder, 1991b). However, the proximal 11-HSD-distal MR model initially proposed to explain the protector function of 11-HSD proved to be less and less adequate as it was reexamined. The great physical distance between 11-HSD and MR appeared to result in an inefficient functional unit. Furthermore, that all available corticosteroid must be inactivated by 11-HSD, implicit in the model, was an untested assumption. The functions of renal GR and the possibility of a functional link between active (steroid bound) GR and the expression of MR remained to be investigated.

The requirement of the original model that access of aldosterone to MR could be achieved only if the tubular filtrate were completely cleansed of glucocorticoid was an extremely stringent one. In order to accommodate this requirement, it was proposed that 11-HSD is strategically distributed along the nephron in order to oxidize residual glucocorticoid. Bonvalet *et al.* (1990) found 11-HSD activity in the distal as well as the proximal portions of the rabbit kidney tubule. Possibly, it was suggested, 11-HSD and MR may coexist in distal cells (Naray-Fejes-Toth *et al.*, 1991; Stewart *et al.*, 1991; Bonvalet, 1991). Gradient fractionation of rat kidney tubules indicated that 11-HSD was indeed in the distal as well as proximal regions (Edwards *et al.*, 1988). The immunohistochemical studies that led to the conclusion that 11-HSD was localized solely in the proximal region of the nephron were in obvious conflict with the enzyme activity data (Rundle *et al.*, 1989a; Castello *et al.*, 1989). The lack of immunoreaction of the distal tubular enzyme with rat liver 11-HSD antibody could be explained by assuming that the enzyme was not easily accessible to antibody because of a transcellular barrier, or was a distantly related antigen. Naray-Fejes-Toth *et al.* (Naray-Fejes-Toth and Fejes-Toth, 1990; Bonvalet *et al.*, 1990), using rabbit kidney cortical collecting tubules isolated by solid-phase immunoadsorption, conclusively showed that there was 11-HSD activity in this region that did not react with antibody on Western blot analysis.

Naray-Fejes-Toth *et al.* (1991) found that the level of 11-HSD activity in monolayer preparations of CCD cells was sufficient to completely convert corticosterone to 11-dehydrocorticosterone in a single pass through the membrane. Therefore, in an individual MR-containing cell, there may be enough 11-HSD to inactivate glucocorticoid completely, thus satisfying the requirement that aldosterone bind MR unencumbered by competing steroids. Differences were also noted in the distribution of 11-HSD in the renal cortex and medulla

(Castello *et al.*, 1989). Consistent with the above postulated autocrine role, the 11-HSD of the distal tubule was more active than the proximal tubule (Edwards *et al.*, 1988; Castello *et al.*, 1989; Bonvalet *et al.*, 1990). A schematic view of the current understanding of corticosteroid associated interactions in normal kidney is shown in Fig. 5.

Evidence that salt metabolism may be mediated through GR as well as MR have been presented by Naray-Fejes-Toth and Fejes-Toth (1990) and Funder *et al.* (1990). The following observations support this conclusion: (*a*) AME patients are more sensitive to cortisol than aldosterone in terms of increased blood pressure and sodium retention; (*b*) in pseudohypoaldosteronism, a condition characterized by low or no MR, the electrolyte effect of cortisol results in part from occupancy of GR; (*c*) RU 28362, a GR-specific glucocorticoid that does not bind MR, affects electrolyte excretion via GR; (*d*) RU 28318, a specific MR antagonist, does not diminish the electrolyte effect of RU 28362; (*e*)

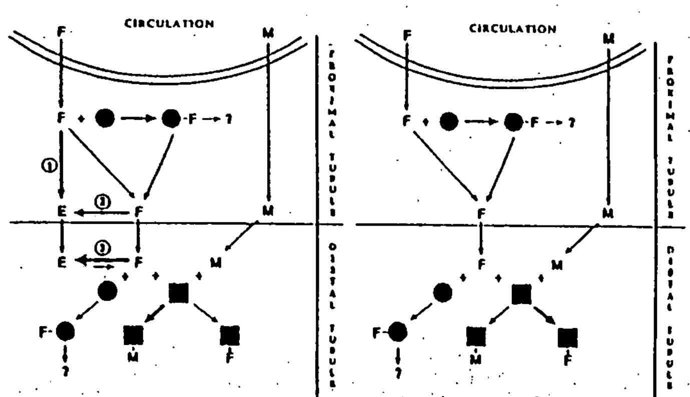


FIG. 5. A current view of corticosteroid associated interactions in normal and 11-HSD-deficient kidney. Heavy arrows indicate dominant pathways. (Left) Normal kidney. Cortisol (F) in the proximal (and possibly distal) tubule mediates glucocorticoid dependent events via glucocorticoid receptor (GR). (●) The level of F available to GR is mediated by 11-HSD (1). Steroid dissociated from GR is oxidized to cortisone (E) (2) to prevent its reentry into the system. F entering from the proximal tubule and other sources may compete with aldosterone (M), for mineralocorticoid receptor (MR). (■) This competition is prevented by oxidation of F to E (3) in the distal tubule and cortical collecting duct. (Right) 11-HSD-deficient kidney. In the absence of functioning 11-HSD, cortisol cannot be oxidized and accumulates, preferentially binding to MR, displacing M, and initiating a sequence of aldosterone-mimetic events.

immunodissected rabbit cortical connecting tubule cells responded similarly to aldosterone, dexamethasone and RU 28362; (*f*) The glucocorticoid receptor antagonist RU 486 blocked the effect of RU 28362, but the MR antagonist ZK 91587 did not; (*g*) kaluresis caused by cortisol is blocked by RU 486 (Clore *et al.*, 1988). Localization of 11-HSD mRNA by *in situ* hybridization using a cRNA probe (Agarwal *et al.*, 1989) indicated its location in the proximal tubules and in the cortical and medullary collecting tubules, a finding that accords with the enzyme distribution studies. The presence of multiple 11-HSD mRNA species in kidney is consistent with the possibility of a heterogeneous population of 11-HSD proteins that may be generated from them, some of which may be recognized by 11-HSD antibody (Krozowski *et al.*, 1990). These results also indicate that the variant forms of 11-HSD that have been proposed may be generally similar in structure.

4. *Licorice, Hypertension, and Kidney Function*

a. The Active Agent of Licorice. Valuable evidence supporting the role of 11-HSD in kidney function emerged from studies on the pharmacological behavior of licorice, a flavoring agent extracted from the roots of *Glycyrrhiza glabra*. Licorice has been used as a medicine and condiment for at least 5000 years (Davis and Morris, 1991). Glycyrrhetinic acid (GA), its active ingredient, is a cyclic triterpene whose fused ring structure, illustrated in Fig. 6, closely resembles that of the glucocorticoids. A synthetic agent developed for the treatment for gastric and duodenal ulcers, carbenoxolone (CA), is the 3-*O*-β-carboxypropionyl ester of glycyrrhetinic acid. Ingestion of either GA or CA causes

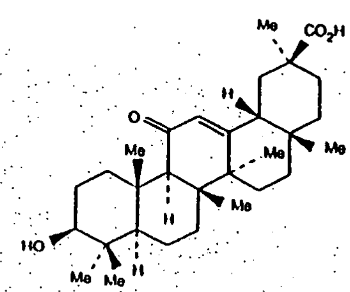


FIG. 6. Glycyrrhetinic acid (GA).

clinical effects that resemble those of aldosterone excess, including hypertension, hypokalemia, edema, polyuria, polydipsia, heart failure, and muscle weakness (Pinder *et al.*, 1976; Baron, 1983; Werning *et al.*, 1971).

b. Possible Explanation of Licorice Actions. Reevers (1948) first documented the aldosterone mimetic behavior of GA. Explanations for its properties have included suggestions that it (*a*) stimulates aldosterone production; (*b*) displaces aldosterone from nonspecific binding sites, thus increasing its effective concentration (Humphrey *et al.*, 1979; Porter, 1970); (*c*) demonstrates intrinsic mineralocorticoid activity; or (*d*) potentiates the effects of aldosterone (Humphrey *et al.*, 1979; Armanini *et al.*, 1989b). All except the last two are unlikely mechanisms (Hausmann and Tarnoky, 1968; Porter, 1970). Evidence for binding of glycyrrhetinic acid to kidney mineralocorticoid receptors was presented by Ulmann *et al.* (1975), Armanini *et al.* (1983), Takeda *et al.* (1987), and Hayashi *et al.* (1984). There is general agreement that binding of GA to MR is about 1/10,000 that of aldosterone based on competitive binding studies. It is unclear whether significant binding to human or rat kidney MR occurs even under the conditions of massive GA or CA intake. In a toad bladder model, 2.5×10^{-6} *M* CA had no effect on short-circuit current over 360 min of exposure (Gaeggeler *et al.*, 1989). Humphrey *et al.* (1979) found that CA did not displace [^{3}H]aldosterone from rat kidney nuclei. The reason may be, as Armanini *et al.* (1989a) suggest, that binding of CA to MR requires its prior hydrolysis to GA.

It has been estimated that subjects consuming 100 to 200 g of licorice per day have total circulating plasma GA levels of 80 to 480 ng/ml (Hughes and Cowles, 1977; Stewart *et al*, 1987). The concentration of free circulating GA is lower, since 95% of GA is bound to plasma proteins (Ishida *et al.*, 1988). Thus, the concentration of GA potentially accessible to MR is too low to measurably bind to the receptor under physiological conditions. It is, however, theoretically possible that specific ligand–receptor interaction may lead to some responses resembling that of the binding of mineralocorticoid. The availability of radioactive glycyrrhetinic acid (Kanaoka *et al.*, 1988) should make it possible to determine whether its interaction with MR leads to nuclear translocation.

Additional evidence that cannot currently be reconciled with the postulated mineralocorticoid-mimetic behavior of GA is the observation that the effectiveness of GA is abolished in adrenalectomized rodents (Card *et al.*, 1953; Girerd *et al.*, 1960) and humans (Borst *et al.*, 1953; Elmadjian *et al.*, 1956) and is restored when glucocorticoids are administered (Borst *et al.*, 1953). The results indicate that a secretory

product of the adrenal cortex is an essential participant of GA action. Normal individuals ingesting glycyrrhetinic acid under controlled conditions for brief periods of time (3–10 days) showed significant decrease in cortisol oxidation to 11-oxo metabolites (MacKenzie *et al.*, 1990), a finding consistent with an inhibitory effect on 11-HSD (Mattingly *et al.*, 1970; Chen *et al.*, 1990; Ojima *et al.*, 1990).

c. Glycyrrhetinic Acid and Other Inhibitors of Renal 11-HSD. Other agents affect the activity of renal 11-HSD. The inhibition of 11-HSD by gossypol, a potential male contraceptive agent extracted from cottonseed oil, resembles that of GA and CA. This observation has led to the suggestion that the hypokalemia observed in men taking this agent has the same cause as that of men ingesting licorice (Sang *et al.*, 1991). Hierholzer and co-workers (1990b) have found that bile acids, though of low inhibitory potency, are present in the human circulation at concentrations that indicate that they have the potential to modulate 11-HSD activity.

Touitou *et al.* (1984) have made the surprising observation that trilostane, a cyanoketone derivative known to inhibit 3β-hydroxysteroid dehydrogenase, increased 11β-hydroxy oxidation in sheep liver homogenates, a phenomenon that may be species specific. Perschel *et al.* (1991) found that pooled rabbit bile at low concentrations increased rat renal 11-HSD. Whether these examples represent stimulation of 11-HSD, as the authors suggest, or an expression of the ability of $P450_{11\beta}$ to catalyze the oxidation of cortisol to cortisone (Suhara *et al.*, 1986) remains undecided.

In a recent study, GA and CA were found to be extremely potent inhibitors of 11-HSD in isolated rat kidney microsomes, with K_i values of 3 n*M* (Monder *et al.*, 1989). In the range 1 to 20 n*M*, reductase was inhibited poorly (Monder *et al.*, 1989; Hierholzer *et al.*, 1991). Glycyrrhetinic acid is the most powerful known inhibitor of 11-HSD (Monder *et al.*, 1989), but it is 10-fold less potent in intact cells. The basis for this difference is unknown. A transmembrane barrier to GA or sequestration to proteins and other macromolecules has been suggested (Monder, 1991c).

The toad bladder, the amphibian counterpart of the nephron, has proven to be a useful model for studying the pharmacological action of CA and GA on the kidney tubule. Using this system, Gaeggeler *et al.* (1989) and Brem *et al.* (1989, 1991) have shown that CA allows corticosterone to be as potent as aldosterone in eliciting the mineralocorticoid response, in accord with the proposed role of CA as an inhibitor of 11-HSD.

d. Glycyrrhetinic Acid: An Inhibitor of Broad Specificity. It is note-

worthy that Morris *et al.* have shown that the metabolism of aldosterone is slowed by glycyrrhetinic acid, a potent inhibitor of cytosolic 5β-reductase and microsomal 3β-hydroxysteroid dehydrogenase (Latif *et al.*, 1990; Tamura *et al.*, 1979; Yoshida *et al.*, 1992). By slowing the rate of inactivation of aldosterone and 11-deoxycorticosterone, these agents potentiate the activity of mineralocorticoids. The two-pronged effect of GA and CA on mineralocorticoid and 11β-hydroxysteroid metabolism would therefore provide a mechanism for inactivating glucocorticoids and simultaneously enhancing the activity of mineralocorticoids.

There are other ways in which GA or CA can affect renal function (Monder, 1991c). Indirect evidence suggests that GA may inhibit glucuronide formation, since it increases the proportion of unconjugated cortisol in urine of people given massive doses of licorice (equivalent to 0.7 to 1.4 g of GA per day for 1–4 weeks) (Epstein *et al.*, 1978). A possible direct effect of glycyrrhetinic acid on (Na^+ – K^+) ATPase (Itoh *et al.*, 1989; Baron and Greene, 1986) may account for some of the effects of GA on the kidneys of adrenalectomized animals. The combined effects of GA on glucocorticoid oxidation at C-11, A-ring reduction, and excretion of unconjugated steroids bear a striking resemblance to the metabolic changes characteristic of AME (Monder *et al.*, 1986). The possibility that an endogenous glycyrrhetinic acid-like compound contributes to the pathology of AME cannot be excluded.

B. The Vascular Bed

It has been known for about 50 years that adrenocortical hormones influence the behavior of the peripheral blood vessels (Swingle and Remington, 1944). These influences include alterations in intra- and extracellular levels of Na^+ and K^+ critical for maintaining vascular tone (Zweifach *et al.*, 1953), and maintenance of the sensitivity of the peripheral vasculature to pressor agents (Darlington *et al.*, 1989; Grunfeld and Eloy, 1987; Ashton and Cook, 1952). These and other effects (Moura and Worcel, 1984; Nichols *et al.*, 1983, 1984; Jazayeri and Meyer, 1988; Haigh and Jones, 1990; Yasunari *et al.*, 1989) are mediated by MR and GR in vascular smooth muscle (VSM) cells. The presence of MR and GR in vascular smooth muscle provides evidence of direct action of corticosteroids on the arterial wall affecting muscle tone and responsiveness to humoral and neurogenic vasoconstrictive stimuli (Kornel *et al.*, 1975; Onoyama *et al.*, 1979).

The whole arterial tree appears to be a target organ for both mineralocorticoids and glucocorticoids (Kornel *et al.*, 1982). There is evidence that the effects of both steroid classes on vascular tissue proceed by

independent processes (Jazayeri and Meyer, 1988, 1989). Vascular smooth muscle cells in culture are affected differently by mineralocorticoid and glucocorticoids. The glucocorticoid effects are blocked by RU 486, indicating GR dependence (Kornel, 1988; Nichols *et al.*, 1985; Meyer and Nichols, 1981). High levels of glucocorticoids could, by binding both the MR and GR, contribute to the pathogenesis of essential hypertension by stimulating vasoconstriction. Funder *et al.* (1989) found that MR of the mesenteric vascular arcade is aldosterone specific *in vivo*. They suggested that, as in the kidney, 11-HSD may mediate the selective mineralocorticoid response. Funder *et al.* (1989) and Walker *et al.* (1991) have confirmed the original report of Kornel *et al.* (1982) that the vessels of the circulatory system express 11-HSD activity.

The enzyme appears to be predominantly in the smaller vessels, a finding that has been interpreted to indicate that by catalyzing the reversible inactivation of glucocorticoids, it modulates tone in the peripheral resistance beds and thereby influences blood pressure. Alternately, as occurs in the brain (see later), the availability of NADP may affect 11-HSD activity. There appears to be insufficient NADP in VSM cells to fully activate the the available 11-HSD, thus making the nucleotide a limiting factor in the expression of enzyme activity. Consistent with the proposed role of 11-HSD, the enzyme and VSM corticosteroid receptors are colocalized, indicating that the regulation of GR–steroid interaction occurs by an autocrine mechanism.

C. The Skin

The modulation of corticosteroid effects by 11-HSD appears to extend to the superficial peripheral vessels. The potency of topical corticosteroid on suppression of the inflammatory response is determined in part by its local persistence; oxidative inactivation by dermal 11-HSD diminishes its effectiveness. It has been proposed that the vasoconstrictor action of corticosteroid contributes to the potentiation of its action, by preventing its loss. Thus, dermal 11-HSD, which accelerates the destruction of inactivation of the steroid in skin, would diminish its topical effectiveness. Consequently, inhibition of 11-HSD activity in target tissues should potentiate the local action of glucocorticoids.

This concept has been put to the test by Teelucksingh *et al.* (1990) who investigated the activity of hydrocortisone on skin. Topical application of glycyrrhetinic acid inhibited dermal 11-HSD, reducing inactivation of cortisol by skin, prolonging and enhancing its topical anti-inflammatory activity. It has been proposed that this property of GA and CA explains their beneficial effects in inflammatory cut-

aneous disorders (Colin-Jones, 1957). However, recent studies have shown that 11-oxoreductase exceeds 11β-dehydrogenase activity in human skin fibroblasts. Whether this is due to the intrinsic character of the skin enzyme or to another rate-limiting step, such as lack of pyridine nucleotide (as is found in brain), is not known. These observations suggest that in human skin the preferred direction of corticosteroid metabolism is reductive (Hammami and Siiteri, 1991; Monder *et al.*, 1986) and, therefore, that the anti-inflammatory effects of topical application of GA on human skin cannot be fully explained by the inhibition of 11-HSD.

D. The Nervous System

1. *Neural 11-HSD*

Interest in the metabolism of corticosteroids in brain and pituitary evolved simultaneously with the recognition of the importance of steroids on brain function (Woodbury, 1958), on the one hand, and the importance of neuroendocrine influences on steroid secretion, on the other. Soon after cortisol had been isolated from human nerve tissue (Touchstone *et al.*, 1963), evidence for the oxidation of corticosteroids to 11-dehydrocorticosteroids by brain tissue was obtained for rat (Peterson *et al.*, 1965; Sholiton *et al.*, 1965), mouse (Grosser, 1966; Tye and Burton, 1980), dog (Miyabo *et al.*, 1973; Eik-Nes and Brizzee, 1965), and primate (Grosser and Axelrod, 1968). Despite the fact that the presence of 11-HSD in nervous tissue had been known for many years, its possible function in the central nervous system has only recently come under investigation. The working assumption is that brain 11-HSD plays an important role in the expression of glucocorticoid-dependent processes.

2. *Receptor-Mediated Selectivity of Corticosteroid Effects*

As with kidney, central MR and GR mediate corticosteroid-specific effects. Neural MR, with properties identical to those of the renal mineralocorticoid binder (Tashima *et al.*, 1989), interacts with corticosterone (or cortisol) and aldosterone with comparable affinity, and binds dexamethasone, a synthetic glucocorticoid, much less efficiently (Beaumont and Fanestil, 1983; Krozowski and Funder, 1983; Wrange and Yu, 1983). The classic glucocorticoid receptor also uses corticosterone as ligand, but prefers dexamethasone. The equivalent affinity of MR for corticosterone and aldosterone in the rat brain contrasts sharply with the clear preference of the receptor for aldosterone in the kidney. It therefore follows that the overwhelmingly greater concentration of corticosterone (10^2- to 10^3-fold that of aldosterone) in the circulation of the rat would result in MR saturated with and largely dependent for its activity on the circulating corticosterone. The system would thus be insensitive to aldosterone, leading to the conclusion that aldosterone can have no effect on brain function.

There is strong evidence for selective aldosterone effects in the central nervous system. It is known that rat brain takes up both aldosterone and corticosterone, with a similar regional distribution. Highest uptake occurs in the hippocampus, septum, and amygdala (Birmingham *et al.*, 1979; Gerlach and McEwen, 1972; Moguilewsky and Raynaud, 1980). The analysis of brain receptor distribution using RU 26988 (a pure glucocorticoid) and RU 28318 and RU 26752 (pure antimineralocorticoids) (Coirini *et al.*, 1985) has led to the conclusion that there are only the two receptor subtypes, MR and GR. These are distributed in neurons and glial cells (Bohn *et al.*, 1991). Despite the 100- to 1000-fold excess of corticosterone over aldosterone in the circulation (Eilers and Peterson, 1964), MR receptors recognize aldosterone in the presence of corticosterone in signaling changes in salt balance, and this effect persists at physiological levels of both mineralocorticoid and glucocorticoid (McEwen *et al.*, 1986; Forman and Mulrow, 1973; Fregly and Rowland, 1985).

Intracerebroventricular (ICV) administration of aldosterone to heminephrectomized rats caused elevated blood pressure. The effect was blocked by the mineralocorticoid receptor antagonist RU 28318. Corticosterone could not replace aldosterone, nor could systemic administration of steroids reproduce these effects (Gomez-Sanchez, 1991). The hypertension induced by aldosterone administered ICV requires corticosterone, for the effect is prevented in bilaterally adrenalectomized rats, and restored by exogenous corticosterone (Gomez-Sanchez, 1991). Arriza and Evans found in a cotransfection assay that MR was more sensitive to mineralocorticoid than to glucocorticoids, despite equivalent binding affinities (Arriza *et al.*, 1988). It has been reported that corticosteroids differentially modulate nutrient intake in rats through central receptor-mediated processes. Implant of aldosterone in the paraventricular nucleus of adrenalectomized rats stimulated ingestion of fat; corticosterone stimulated carbohydrate intake (Tempel and Liebowitz, 1989).

The interrelationships between GR, MR, and corticosteroids in the central nervous system are complex. Receptor specificity varies in ways that are not immediately obvious. In early studies, the differential binding of corticosterone to receptors suggested that there may be

three receptor types in the nervous system: the classical GR and MR, and a corticosterone binding subset of MR termed CR. Binding studies with corticosterone and aldosterone *in vitro* showed no distinction between CR and MR, and the former term was abandoned, since its retention obscured the question of the specificity-conferring mechanism. To illustrate this point, MR in the circumventricular region is aldosterone selective; MR in the neurons of the limbic region is corticosterone selective. This selectivity is reflected throughout the nervous system and shows up as differential retention of corticosterone and aldosterone in different subregions.

a. Antagonistic and Synergistic Mechanisms. DeKloet and co-workers have developed a functional rationale for the preferential binding of glucocorticoids to both GR and MR under normal physiological conditions (de Kloet and Reul, 1987). They have shown that the circulating concentration of corticosterone results in 80 to 90% occupancy of cerebral MR. This generates a baseline level of continuously activated receptor that serves to monitor and interpret the animal's external environment. At basal levels of circulating corticosterone, specifically at the diurnal trough, the levels of occupancy of GR is low; under stress, or to a lesser extent at the diurnal peak, the level of circulating corticosterone increases, leading to GR occupancy, generating a negative feedback on stress-activated brain mechanisms. There are, thus, reciprocal balancing tonic-activating actions and feedback-damping mechanisms. This continuum reflects corticosteroid concentration, and the sequential, selective occupancy of corticosterone to MR and GR. Evans and Arriza (Arriza *et al.*, 1988) have suggested that MR and GR act as a binary response system for corticosterone. Their model depends on the coordinated synergistic interaction of MR and GR with overlapping sets of genes, the magnitude of the response being dependent on the circulating glucocorticoid levels. This model may be compared with the coordinated antagonistic MR- and GR-mediated effects proposed by DeKloet. Van den Berg *et al.* (1990) suggest that central MR and GR mediate opposing effects of glucocorticoids and mineralocorticoids on blood pressure, consistent with other evidence that MR and GR in the brain mediate reciprocal neurochemical, neuroendocrine, and behavioral responses.

b. Hippocampal Degeneration. The hippocampus illustrates the importance of regional optimization of corticosteroid concentration. This organ contains the highest concentration of MR in the central nervous system (Reul and de Kloet, 1985; Krozowski and Funder, 1983; de Kloet *et al.*, 1984). It is extremely vulnerable to corticosteroid hormones. Chronic glucocorticoid exposure coupled with other chronic

insults (e.g., aging, ischemia) results in the degeneration of specific hippocampal (CA1, CA3) fields (Sapolsky and Pulsinelli, 1985; Dokas, 1990). Glucocorticoid absence also results in specific hippocampal degeneration (Sloviter *et al.*, 1989). There must therefore be a mechanism to maintain glucocorticoids (e.g., corticosterone) in specific hippocampal fields within a defined range of intracellular concentration. Possibly, the survival of these cells requires a persistent occupation of the MR or GR by steroid ligand at some tonic level (Sloviter *et al.*, 1989). That the effects are direct is supported by the observation that cells in culture as well as in intact brain are sensitized by excess glucocorticoid (Masters *et al.*, 1989; Freshney *et al.*, 1980).

c. Selectivity of Brain Corticosteroid Receptors: Proposed Role of 11-HSD. The source of the regional selectivity of brain receptors for mineralo- or glucocorticoid has not yet been determined. The evolution of hypotheses designed to investigate this question has paralleled those proposed for the kidney. For several years, the only serious contender for a selection mechanism was CBG, but this was withdrawn for the same reason as in the kidney (de Kloet and Reul, 1987; Funder, 1986); i.e., selectivity was not altered in animals with little or no circulating CBG. The suggestion that receptor selectivity was mediated by the local action of 11-HSD on glucocorticoids, similar to a parallel process in kidney, initiated a series of promising investigations. The starting point was the hypothesis that the excess corticosterone in blood entering cells represents an impediment to the ability of aldosterone to gain access to the MR in the absence of 11-HSD.

Oxidation of corticosterone to 11-dehydrocorticosterone by 11β-dehydrogenase occurred in widely distributed regions of the brain. Activities were highest in the hippocampus and cortex (Lakshmi *et al.*, 1991; Moisin *et al.*, 1990a), an observation that was confirmed by immunohistochemical staining of brain regions with 11β-dehydrogenase antibody (Lakshmi *et al.*, 1991; R. Rousseau *et al.*, 1972) and by *in situ* hybridization (Moisin *et al.*, 1990a) using cDNA corresponding to rat liver 11β-dehydrogenase (Agarwal *et al.*, 1989).

There is as yet no direct experimental evidence to show that 11-HSD is the selection mechanism for brain receptor. Correlation of 11-HSD activity and intensity of immunoreactive labeling is consistent with a protective mechanism. Using neuronal and glial markers to measure the distribution of 11-HSD-like antigen, it was found that 11-HSD was distributed in the hippocampus in the CA1-4 regions and the dentate gyrus. The distribution in the hippocampus and cortex coincided with the distribution of MR. Neuronal 11-HSD was found throughout the cell body and its projections. Consistent with the hypothesis that 11-

HSD is the selection mechanism for MR, receptor and enzyme were located within the same cell (Sakai *et al.*, 1990). The distribution was heterogeneous, with some neurons that contained MR showing no detectable 11-HSD immunoreactivity. The distribution studies suggest that the selection mechanism of 11-HSD protection of MR is retained within the individual neuron. The observation that the enzyme is localized in the neuronal nuclei and the discovery that in all brain regions investigated 11-HSD is found in glial cells suggest that its functions are complex.

In the hippocampus, as in other brain regions, gluco- and mineralocorticoids must both be present in some crucial, though as yet unknown, relationship for optimal function to occur. A neuron containing both GR and MR must be able to manipulate both glucocorticoid and mineralocorticoid levels to permit functionally adequate binding to the available receptors. This may require that corticosterone concentrations be adjusted to permit its occupancy of MR and/or GR in a way that is in accord with the needs of the cell, or alternatively, to permit glucocorticoid metabolism to proceed extensively in order for the MR to bind aldosterone. How 11-HSD activity is controlled to permit optimal neuronal function is not known. Several mechanisms are possible: (*a*) controlled synthesis and inactivation of enzyme; (*b*) control of activity based on availability of cofactor; (c) reversibility of enzyme, permitting net oxidation or reduction of 11-oxygenated steroid to occur.

In some regions of the brain, 11-HSD may mediate GR-dependent events. Cerebellum contains no measurable MR, but does have well-defined GR; 11-HSD is expressed as high activity accompanied by high levels of 11-HSD mRNA (Moisin *et al.*, 1990a). If 11-HSD serves any receptor-related function in cerebellum, it must only influence GR-dependent events. It has been suggested that 11-HSD may control glucose metabolism in the brain via GR. Inhibition of 11-HSD by glycyrrhetinic acid increased steroid-dependent uptake of 2-[^{14}C] deoxyglucose in the arcuate nucleus, proptic area, cortex, hippocampus, and paraventricular nucleus (Seckl *et al.*, 1991). Glial cells contain GR (McGinnis and de Vellis, 1981), but no MR, respond to glucocorticoids, and contain 11-HSD. There are thus several examples of cell types in which the resident 11-HSD may serve cell-specific functions depending on their receptor content.

E. Leydig Cells, Stress, and 11-HSD

An extensive literature has accumulated that shows that the testis synthesizes less testosterone when exposed to pharmacological levels of circulating corticosteroid, and that the diminished responses are receptor mediated (Phillips *et al.*, 1989). Extending the idea first proposed for kidney function, it was suggested that 11-HSD protects the production of testosterone by Leydig cells against the inhibitory effects of glucocorticoids. That testicular 11-HSD is restricted to the Leydig cells is consistent with this hypothesis.

By inactivating cortisol (corticosterone in the rat) 11-HSD acts as an enzymatic barrier. This testicular barrier is overwhelmed when the level of circulating glucocorticoid exceeds a threshold defined by the enzyme's ability to oxidize the steroid. It was recently found that 11-HSD is absent from rat Leydig cells prior to the twenty-fifth postnatal day (Phillips *et al.*, 1989; Haider *et al.*, 1990). This observation suggested that prior to 25 days of age, corticosterone cannot be inactivated and thus contributes to the prepubertal suppression of testosterone production. Subsequently, as enzyme is expressed and corticosteroid is oxidized, inhibition of testosterone is overcome. As the animal ages, it is only possible to inhibit testosterone production with amounts of cortisol and corticosterone that exceed the oxidative capacity of 11-HSD, or by glucocorticoid analogs, such as dexamethasone, that are poor substrates for the enzyme. The 11-HSD inhibitor carbenoxolone increases the testosterone suppressive effects of corticosterone, an effect in accord with predictions (Abayasekara *et al.*, 1990). The synthetic glucocorticoid dexamethasone inhibits testosterone secretion by Leydig cells, but since, unlike corticosterone, it is not a substrate of 11-HSD, its effect is not increased by 11-HSD inhibitors (Monder *et al.*, 1992). The mineralocorticoid aldosterone has no effect on testosterone production (Abayasekara *et al.*, 1990; Monder *et al.*, 1992), since Leydig cells have no MR (R. R. Sakai, M. Hardy, and C. Monder, unpublished observations).

F. Mammary Gland

In the mammary gland, glucocorticoids are required for the synthesis of casein, lactalbumin, and other proteins, through a GR-dependent process (Jahn *et al.*, 1987). Quirk *et al.* (1990a) have found 11-HSD in the epithelial and adipose tissue of pregnant and lactating mammary gland of rats. The enzyme is 20-fold higher in adipocytes than epithelial cells and diminishes in both cell types as pregnancy progresses to reach low levels in lactating glands. The authors propose that 11-HSD decreases local concentration of corticosterone by the formation of the inactive 11-dehydrosteroid metabolite, and thus prevents premature milk production (Quirk *et al.*, 1990a,b). The presence of MR in breast tissue (Quirk *et al.*, 1983) suggests that the role of 11-HSD in

the mammary gland may involve the participation of corticosteroids on salt and water metabolism (Molina *et al.*, 1990) as well as on milk protein production.

X. Epilogue

In this article, we have attempted to provide a historical perspective into the conceptual evolution of 11β-hydroxysteroid dehydrogenase from its pedestrian origin as an enzyme that catalyzes reversible inactivation of corticosteroids to its currently more prestigious role as mediator of steroid–receptor interactions. The recent surge of interest in 11-HSD was powered by two factors. The first was the recognition of clinical disorders whose symptomatology could be rationalized as being due to defects in 11-HSD expression. The second was the development of the tools—antibodies, cDNA—that facilitated exploration of the enzyme at the molecular level. With the use of these probes, investigators have developed hypotheses implicating the interplay of corticosteroids, 11-HSD, and steroid receptors in the etiology of juvenile hypertension, and in normal renal function. The success of this endeavor has inspired further exploration of the possibility that the principles that emerged from the study of the kidney also apply to other organs. The current state of these investigations has been summarized in this article. The significance of 11-HSD as a mediator of steroid–receptor interaction cannot, however, be unique. The concepts emerging from studies with this corticosteroid-metabolizing enzyme ought to apply as well to enzymes participating in the metabolism of other classes of steroid. Thus, there is reason to believe that the answers emerging from the questions posed in this article may find application elsewhere in steroid biology.

Acknowledgments

We thank Maria I. New and Phyllis Speiser for their contributions to this work, and Dr. Cedric Shackleton for his help in compiling Table VIII. Original research described was supported by U.S. Public Health Services Grants DK 37094 and DK42169 and March of Dimes Grant 6-609.

References

Abayasekara, D. R. E., Band, A. M., and Cooke, B. A. (1990). Inhibition of Leydig cell steroidogenesis by adrenal steroids: Specificity, time dependency and effects of an 11β-dehydrogenase inhibitor. *J. Endocrinol.* 124, Suppl. Abst 73.

Abramovitz, M., Branchaud, C. L., and Murphy, B. E. P. (1982). Cortisol-cortisone interconversions in human fetal lung: Contrasting results using explant and monolayer cultures suggest that 11β-hydroxysteroid dehydrogenase (EC 1.1.1.46) comprises two enzymes. *J. Clin. Endocrinol. Metab.* 54, 563–568.

Abramovitz, M., Carriero, R., and Murphy, B. E. P. (1984). Investigation of factors influencing 11β-hydroxysteroid dehydrogenase (EC 1.1.1.146) activity in midgestation human fetal lung monolayer and explant cultures. *J. Steroid Biochem.* 21, 677–683.

Adam, W. R., Funder, J. W., Mercer, J., and Ullick, S. (1978). Amplification of the action of aldosterone by 5α-dihydrocortisol. *Endocrinology (Baltimore)* 103, 465–471.

Agarwal, A. L., Monder, C., Eckstein, B., and White, P. C. (1989). Cloning and expression of rat cDNA encoding corticosteroid 11β-dehydrogenase. *J. Biol. Chem.* 264, 18939–18943.

Agarwal, A. K., Tusie-Luna, M. T., Monder, C., and White, P. C. (1990). Expression of 11β-hydroxysteroid dehydrogenase using recombinant vaccinia virus. *Mol. Endocrinol.* 4, 1827–1832.

Althaus, Z. R., Bailey, J. R., Leakey, J. E. A., and Slikker, W., Jr. (1982). Transplacental metabolism of dexamethasone and cortisol in late gestational age of rhesus monkey (*Macaca mulata*). *Dev. Pharmacol. Ther.* 9, 332–349.

Amelung, D., Hubener, H. J., and Roka, L. (1953a). Enzymatische Umwandlungen von Steroiden III Über die Einfuhrung der 11-Oxygruppe und die Reduktion der 11-Keto gruppe. *Hoppe-Seyler's Z. Physiol. Chem.* 294, 36–48.

Amelung, D., Huebener, H. J., Roka, L., and Meyerheim, G. (1953b). Conversion of cortisone to compound F. *J. Clin. Endocrinol. Metab.* 13, 1125.

Anderson, D. F., Stock, M. K., and Rankin, J. H. G. (1979). Placental transfer of dexamethasone in near term sheep. *J. Dev. Physiol.* 1, 431–436.

Armanini, D., Karbowiak, I., and Funder, J. W. (1983). Affinity of liquorice derivatives for mineralocorticoid and glucocorticoid receptors. *Clin. Endocrinol. (Oxford)* 19, 609–612.

Armanini, D., Strasser, T., and Weber, P. C. (1985). Characterization of aldosterone binding sites in circulating human mononuclear leukocytes. *Am. J. Physiol.* 248, E388–E390.

Armanini, D., Wehling, M., and Weber, P. C. (1989a). Mineralocorticoid effector mechanism of liquorice derivatives in human mononuclear leukocytes. *J. Endocrinol. Invest.* 12, 303–306.

Armanini, D., Scali, M., Zennaro, M. C., Karbowiak, I., Wallace, C., Lewicka, S., Vecsel, P., and Mantero, F. (1989b). The pathogenesis of pseudohyperaldosteronism from carbenoxolone. *J. Endocrinol. Invest.* 12, 337–341.

Arriza, J. L. (1991). Aldosterone action: Perspectives from the cloning of the mineralocorticoid receptor. *Colloq.—Inst. Natl. Sante Rech. Med.* 216, 13–21.

Arriza, J. L., Weinberger, C., Cerelli, G., Glaser, T. M., Handelin, B. L., Housman, D. E., and Evans, R. M. (1987). Cloning of human mineralocorticoid receptor complementary DNA: Structural and functional kinship with the glucocorticoid receptor. *Science* 237, 268–275.

Arriza, J. L., Simerly, R. B., Swanson, L. W., and Evans, R. M. (1988). The neuronal mineralocorticoids receptor as a mediator of glucocorticoid responses. *Neuron* 1, 887–900.

Ashton, N., and Cook, C. (1952). *In vivo* observations of the effects of cortisone upon blood vessels in rabbit ear chambers. *Br. J. Exp. Pathol.* 33, 445–450.

Avery, M. E. (1975). Pharmacological approaches to the acceleration of fetal lung maturation. *Br. Med. Bull.* 31, 13–17.

Baggia, S., Albrecht, E. D., and Pepe, G. J. (1990). Regulation of 11β-hydroxysteroid

dehydrogenase activity in baboon placenta by estrogen. *Endocrinology (Baltimore)* 126, 2742–2748.

Bailey, E., and West, H. F. (1969). The secretion, interconversion and catabolism of cortisol, cortisone and some of their metabolites in man. *Acta Endocrinol. (Copenhagen)* 62, 339–359.

Baird, C. W., and Bush, I. E. (1960). Cortisol and cortisone content of amniotic fluid from diabetic and non diabetic women. *Acta Endocrinol (Copenhagen)* 34, 97–104.

Baker, M. E. (1989). Human placental 17β-hydroxysteroid dehydrogenase is homologous to NodG protein of Rhizobium meliloti. *Mol. Endocrinol.* 3, 881–884.

Baker, M. E. (1990a). Sequence similarity between Pseudomonas dihydrodiol dehydrogenase, part of the gene cluster that metabolizes polychlorinated biphenyls and dehydrogenases involved in metabolism of ribitol and glucitol and synthesis of antibiotics and 17β-oestradiol, testosterone and corticosterone. *Biochem. J.* 267, 839–841.

Baker, M. E. (1990b). A common ancestor for human placental 17β-hydroxysteroid dehydrogenase, *Streptomyces coelicolor* act III protein, and *Drosophila melanogaster* alcohol dehydrogenase. *FASEB J.* 4, 222–226.

Baron, D. N., and Greene, R. J. (1986). Action of compounds with effective in vivo mineralocorticoid activity on ion transport in leucocytes. *Br. J. Clin. Pharmacol.* 21, 27–34.

Baron, J. H. (1983). Side effects of carbenoxolone. *Acta Gastroenterol. Belg.* 46, 469–484.

Barseghian, G., Levine, R., and Epps, P. (1982). Direct effect of cortisol and cortisone on insulin and glucagon secretion. *Endocrinology (Baltimore)* 111, 1648–1651.

Batista, M. C., Mendonca, B. B., Kater, C. E., Arnhold, I. J. P., Rocha, A., Nicolau, W., and Bloise, W. (1986). Spironolactone-reversible rickets associated with 11β-hydroxysteroid dehydrogenase deficiency syndrome. *J. Pediatr.* 109, 989–993.

Baulieu, E. E., and Jayle, M. F. (1957). Etude de l'équilibre entre 11β-hydroxy et 11-cetostéroïdes. I. Mésures de la cortisone, du cortisol et de leurs métabolites tetrahydrogènes urinaires après administration de cortisone, cortisol et ACTH à l'homme. *Bull. Soc. Chim. Biol.* 39, 37.

Baxter, J. D., Schambelan, M., Matulich, D. T., Spindler, B. J., Taylor, A. A., and Bartter, F. C. (1976). Aldosterone receptors and the evaluation of plasma mineralocorticoid activity in normal and hypertensive states. *J. Clin. Invest.* 58, 579–589.

Beaumont, K., and Fanestil, D. D. (1983). Characterization of rat brain aldosterone receptors reveals high affinity for corticosterone. *Endocrinology (Baltimore)* 113, 2043–2051.

Beitins, I. Z., Bayard, F., Ances, I. G., Kowarski, A., and Migeon, C. J. (1972). The transplacental passage of prednisone and prednisolone in pregnancy near term. *J. Pediatr.* 81, 936–945.

Berliner, D. L. (1965). Studies of the mechanisms by which cells become resistant to steroids. *Cancer Res.* 25, 1085–1095.

Berliner, D. L., and Ruhmann, A. G. (1966). Comparison of the growth of fibroblasts under the influence of 11β-hydroxy and 11-keto compounds. *Endocrinology (Baltimore)* 78, 373–382.

Bernal, A. Lopez, and Craft, I. L. (1981). Corticosteroid metabolism in vitro by human placenta, fetal membranes and decidua in early and late gestation. *Placenta* 2, 279–286.

Bernal, A. Lopez, and Turnbull, A. C. (1985). Cortisol metabolism in human placenta and decidua is resistant to anoxia. *Horm. Meta. Res.* 17, 107.

Bernal, A. Lopez, Flint, A. P. F., Anderson, A. B. M., and Turnbull, A. C. (1980). 11β-

hydroxysteroid dehydrogenase activity (E.C. 1.1.1.146) in human placenta and decidua. *J. Steroid Biochem.* 13, 1081–1087.

Bernal, A. Lopez, Anderson, A. B. M., and Turnbull, A. C. (1982). The lack of influence of labor on human placental 11β-hydroxysteroid dehydrogenase activity. *J. Clin. Endocrinol. Metab.* 54, 1251–1254.

Bigger, J. F., Palmberg, P. F., and Becker, B. (1972). Increased cellular sensitivity to glucocorticoids in primary open angle glaucoma. *Invest. Ophthalmol.* 11, 832–837.

Birmingham, M. K., Stumpf, W. S., and Sar, M. (1979). Nuclear localisation of aldosterone in rat brain cells assessed by autoradiography. *Experientia* 35, 1240–1241.

Blair-West, J. R., Coghlan, D. A., Denton, D. A., Goding, J. R., Munro, J. A., Peterson, R. E., and Wintour, M. (1962). Humoral stimulation of adrenal cortical secretion. *J. Clin. Invest.* 41, 1606–1627.

Blunck, W. (1968). Die α-ketolischen cortisol-und corticosteronmetaboliten sowie die 11-oxy und 11-desoxy-17-ketosteroide im urin von kindern. *Acta Endocrinol. (Copenhagen), Suppl.* 134, 9–112.

Bohn, M. C., Howard, E., Vielkind, U., and Krozowski, Z. (1991). Glial cells express both mineralocorticoid and glucocorticoid receptors. *J. Steroid Biochem. Mol. Biol.* 40, 105–111.

Boland, E. W. (1952). Antirheumatic effects of hydrocortisone (free alcohol), hydrocortisone acetate and cortisone (free alcohol) as compared with cortisone acetate. *Br. Med. J.* 1, 559–564.

Bonvalet, J.-P. (1991). Aldosterone-sensitive cells in the kidney: New insights. *News Physiol. Sci.* 6, 201–205.

Bonvalet, J.-P., Doignon, J., Biot-Chabaud, M., Pradelle, P., and Farman, N. (1990). Distribution of 11β-hydroxysteroid dehydrogenase along the rabbit nephron. *J. Clin. Invest.* 86, 832–837.

Borst, J. G. G., Ten Holt, S. P., De Vries, L. A., and Molhuysen, J. A. (1953). Synergistic action of liquorice and cortisone in Addison's and Simmonds' disease. *Lancet* 1, 657–663.

Bradlow, H. L., Zumoff, B., Gallagher, T. F., and Hellman, L. (1968). Tetrahydrocortisol metabolism in man. *Steroids* 12, 303–308.

Brem, A. S., Matheson, K. L., Conca, T., and Morris, D. J. (1989). Effect of carbenoxolone on glucocorticoid metabolism and Na transport in toad bladder. *Am. J. Physiol.* 257, F700–F704.

Brem, A. S., Matheson, K. L., Barnes, J. L., and Morris, D. J. (1991). 11-dehydrocortisone, a glucocorticoid metabolite, inhibits aldosterone action in toad bladder. *Am. J. Physiol.* 261, F873–F879.

Bro-Rasmussen, F., Buus, O., and Trolle, D. (1962). Ratio of cortisone/cortisol in mother and infant at birth. *Acta Endocrinol. (Copenhagen)* 40, 579–583.

Brown-Sequard, C. E. (1856). Recherches expérimentales sur la physiologie des capsules surrénales. *C. R. Hebd. Seances Acad. Sci.* 43, 422.

Burstein, S., Savard, K., and Dorfman, R. I. (1953). The in vivo metabolism of hydrocortisone. *Endocrinology (Baltimore)* 53, 88–97.

Burton, A. F. (1965). Inhibition of 11β-hydroxysteroid dehydrogenase activity in rat and mouse in vitro and in vivo. *Endocrinology (Baltimore)* 77, 325–331.

Burton, A. F., and Anderson, F. H. (1983). Inactivation of corticosteroids in intestinal mucosa by 11β-hydroxysteroid NADP oxidoreductase (EC1.1.1.146). *Am. J. Gastroenterol.* 78, 627–631.

Edwards, C. R. W., Stewart, P. M., Burt, D., Brett, L., McIntyre, M. A., Sutanto, W. S., de Kloet, E. R., and Monder, C. (1988). Localization of 11β-hydroxysteroid dehydrogenase-tissue specific protector of the mineralocorticoid receptor. *Lancet* 2, 986–989.
Edwards, C. R. W., Burt, D., and Stewart, P. M. (1989). The specificity of the human mineralocorticoid receptor: Clinical clues to a biological conundrum. *J. Steroid Biochem.* 32, 213–216.
Eik-Nes, K. B., and Brizzee, K. R. (1965). Concentration of tritium in brain tissue of dogs given [1,2-³H] cortisol intravenously. *Biochim. Biophys. Acta* 97, 320–333.
Eilers, E. A., and Peterson, R. E. (1964). Aldosterone secretion in the rat. *In* "Aldosterone" (E. E. Baulieu and P. Robel, eds.), pp. 251–264. Blackwell, Oxford.
Eisenstein, A. B. (1952). Steroid compounds resulting from incubation of cortisone with surviving liver slices. *Science* 116, 520–521.
Elmadjian, F., Hope, J. M., and Pincus, G. (1956). The action of mono-ammonium glycyrrhizinate on adrenalectomised subjects and its synergism with hydrocortisone. *J. Clin. Endocrinol. Metab.* 16, 338–349.
Endahl, G. L., and Kochakian, C. D. (1962). Partial purification and further characterization of the triphosphopyridine nucleotide specific C_{19}-17β-hydroxysteroid dehydrogenase of guinea pig liver. *Biochim. Biophys. Acta* 62, 245–250.
Endahl, G. L., Kochakian, C. D., and Hamm, D. (1960). Separation of a triphosphopyridine nucleotide-specific from a diphosphopyridine nucleotide-specific 17β-hydroxy (testosterone) dehydrogenase of guinea pig liver. *J. Biol. Chem.* 235, 2792–2796.
Engel, L. L., Carter, P., and Fielding, L. L. (1955). Urinary metabolites of administered corticosterone. I. Steroids liberated by glucuronidase hydrolysis. *J. Biol. Chem.* 213, 99–106.
Epstein, M. T., Espiner, E. A., Donald, R. A., Hughes, H., Cowles, R. J., and Lun, S. (1978). Licorice raises urinary cortisol in man. *J. Clin. Endocrinol. Metab.* 47, 397–400.
Eriksson, H., and Gustafsson, J. A. (1971). Metabolism of corticosterone in the isolated perfused rat liver. *Eur. J. Biochem.* 20, 231–236.
Ernster, L., and Jones, L. C. (1962). A study of nucleoside tri- and diphosphate activities of rat liver microsomes. *J. Cell Biol.* 15, 563–578.
Evans, R. M. (1988). The steroid and thyroid hormone receptor superfamily. *Science* 240, 889–895.
Farman, N., Vandewalle, A., and Bonvalet, J. P. (1983). Autoradiographic determination of dexamethasone binding sites along the rabbit nephron. *Am. J. Physiol.* 244, F325–F334.
Farman, N., Oblin, M. E., Lombes, M., Delahaye, F., Westphal, H. M., Bonvalet, J. P., and Gasc, J. M. (1991). Immunolocalization of gluco- and mineralocorticoid receptors in rabbit kidney. *Am. J. Physiol.* 260, C226–C233.
Fazekas, A. G., Sandor, T., and Lanthier, A. (1970). Conversion of corticosterone to 11-dehydrocorticosterone by adrenal gland preparations of different animal species. *Endocrinology (Baltimore)* 86, 438–440.
Fieser, L. F., and Fieser, M. (1959) "Steroids." Reinhold, New York.
Fiselier, W., Otten, B. J., Monnens, L. A. H., Honour, J. W., and van Munster, P. J. J. (1982). Low-renin, low-aldosterone hypertension and abnormal cortisol metabolism in a 19-month-old child. *Horm. Res.* 16, 107–114.
Fish, C. A., Hayano, M., and Pincus, G. (1953). Conversion of cortisone to 17-hydroxycorticosterone by liver homogenates. *Arch. Biochem. Biophys.* 42, 480–481.

Forman, B. H. and Mulrow, P. J. (1973). Effect of corticosteroids on water and electrolyte metabolism. *In* "Handbook of Physiology" (R. O. Greep and E. B. Astwood, eds.), Sect. 7, Vol. 6, pp. 179–180. Am. Physiol. Soc., Washington, DC.
Fregly, M. J., and Rowland, N. E. (1985). Role of renin-angiotensin-aldosterone system in NaCl appetite of rats. *Am. J. Physiol.* 248, R1–R11.
Freshney, R. I., Sherry, A., Hassanzadah, M., Freshney, M., Crilly, P., and Morgan, D. (1980). Control of cell proliferation in human glioma by glucocorticoids. *Br. J. Cancer* 41, 857–866.
Fukushima, D. K., Gallagher, T. F., Greenberg, W., and Pearson, O. H. (1960). Studies with an adrenal inhibitor in adrenal carcinoma. *J. Clin. Endocrinol. Metab.* 20, 1234–1245.
Funder, J. W. (1986). Adrenocorticoid receptors in the brain. *In* "Frontiers in Neuroendocrinology" (W. F. Ganong and L. Martini, eds.), Vol. 9, pp. 169–189. Raven Press, New York.
Funder, J. W. (1987). Adrenal steroids: New answers, new questions. *Science* 237, 236.
Funder, J. W. (1990a). Target tissue specificity of mineralocorticoids. *TEM* 1, 145–148.
Funder, J. W. (1990b). Corticosteroid receptors and renal 11β-hydroxysteroid dehydrogenase activity. *Semin. Nephrol.* 10, 311–319.
Funder, J. W., Pearce, P. T., Smith, R., and Smith, A. I. (1988). Mineralocorticoid action: Target tissue specificity is enzyme, not receptor, mediated. *Science* 242, 583–585.
Funder, J. W., Pearce, P. T., Smith, R., and Campbell, J. (1989). Vascular Type I aldosterone binding sites are physiological mineralocorticoid receptors. *Endocrinology (Baltimore)* 125, 2224–2226.
Funder, J. W., Pearce, P. T., Myles, K., and Roy, L. P. (1990). Apparent mineralocorticoid excess, pseudohypoaldosteronism and urinary electrolyte excretion: Toward a redefinition of mineralocorticoid action. *FASEB J.* 4, 3234–3238.
Funkenhouser, J. D, Peevy, K. J., Mockridge, P. B., and Hughes, E. R (1978). Distribution of dexamethasone between mother and fetus after maternal administration. *Pediatr. Res.* 12, 1053–1056.
Furguson, M. M., and MacPhee, G. B. (1975). Kinetic study of 11β-hydroxysteroid dehydrogenase in rat submandibular salivary gland. *Arch. Oral Biol.* 20, 241–245.
Gaeggeler, H.-P., Edwards, C. R. W., and Rossier, B. C. (1989). Steroid Metabolism determines mineralocorticoid specificity in the toad bladder. *Am. J. Physiol.* 257, F690–F695.
Gallagher, T. F., Hellman, L., Zumoff, B., and Miller, D. G. (1965). Steroid hormone metabolism in chronic myelogenous leukemia. *Blood* 25, 743–748.
Gerlach, J., and McEwen, B. S. (1972). Rat brain binds adrenal steroid hormone: Autoradiography of hippocampus with corticosterone. *Science* 175, 1133–1136.
Ghosh, D., Weeks, C. M., Grochulski, P., Duax, W. L., Erman, M., Rimsay, R. L., and Orr, J. C. (1991). Three-dimensional structure of holo 3α, 20β-hydroxysteroid dehydrogenase: A member of a short-chain dehydrogenase family. *Proc. Natl. Acad. Sci. U.S.A.* 88, 10064–10068.
Ghraf, R., Vetter, U., Zandveld, J. M., and Schriefers, H. (1975a). Organ specific ontogenesis of steroid metabolizing activities in the rat I. 11β and 17β hydroxysteroid dehydrogenase. *Acta Endocrinol. (Copenhagen)* 79, 192–201.
Ghraf, R., Hoff, H. G., Lax, E. R., and Schriefers, H. (1975b). Enzyme activity in kidney, adrenal and gonadal tissue of rats treated neonatally with androgen or estrogen. *Endocrinology (Baltimore)* 67, 317–326.
Giannopoulos, G. (1974). Uptake and metabolism of cortisone and cortisol by fetal rabbit lung. *Steroids* 23, 845–853.

Giannopoulos, G., Jackson, K., and Tulchinsky, D. (1982) Glucocorticoid metabolism in human placenta, decidua, myometrium and fetal membranes. *J. Steroid Biochem.* 17, 371–374.

Girerd, R. J., Rassnort, C. L., DiPasquale, G., and Kroc, R. L. (1960). Endocrine involvement in licorice hypertension. *Am. J. Physiol.* 198, 718–720.

Gomez-Sanchez, E. P. (1991). What is the role of the central nervous system in mineralocorticoid hypertension?. *Am. J. Hypertens.* 4, 374–381.

Gordon, G. G., and Southren, A. L. (1977). Thyroid hormone effects on steroid hormone metabolism. *Bull. N.Y. Acad. Med.* 53, 241–259.

Gottfried, H. (1964). The occurrence and biological significance of steroids in lower vertebrates. *Steroids* 3, 219–242.

Gray, C. H., Greenaway, J. M., Holness, N. J., and Shaw, D. A. (1962). Metabolism of Cortisol-4-C^{14} in a patient with Cushings Syndrome. *J. Endocrinol.* 24, 199–214.

Grosser, B. I. (1966). 11β-hydroxysteroid metabolism by mouse brain and glioma 261. *J. Neurochem.* 13, 475–478.

Grosser, B. I., and Axelrod, L. R. (1968). Conversion of cortisol to cortisol acetate, cortisone acetate and cortisone by the developing primate brain. *Steroids* 11, 827–836.

Grosso, L. L., and Unger, F (1964). Conversion of pregnenolone and 4-androstene-3β, 17β-diol to testosterone by mouse testes *in vitro*. *Steroids* 3, 67–76.

Grunfeld, J.-P., and Eloy, L. (1987). Glucocorticoids modulate vascular reactivity in the rat. *Hypertension* 10, 608–618.

Guignard-de Maeyer, J. A., Crigler, J. F., and Gold, N. I. (1963). An alteration in cortisol metabolism in patients with Cushing's syndrome and bilateral adrenal hyperplasia. *J. Clin. Endocrinol. Metab.* 23, 1271–1284.

Gunderson, H. M., and Nordlie, R. C. (1975). Carbamyl phosphate: Glucose phosphotransferase and glucose-6-phosphate phosphohydrolase of nuclear membrane. Interrelationships between membrane integrity, enzyme latency and catalytic behavior. *J. Biol. Chem.* 250, 3552–3559.

Gustafsson, J. A., and Gustafsson, S. A. (1974). Delayed expression of neonatal sexual differentiation of corticosteroid patterns in rat bile. *Eur. J. Biochem.* 44, 225–233.

Gustafsson, J. A., and Stenberg, A. (1976). Obligatory role of hypophysis in sexual differentiation of hepatic metabolism in rats. *Proc. Natl. Acad. Sci. U.S.A.* 73, 1462–1465.

Haider, S. G., Passia, D., and Rommert, F. F. G. (1990). Histochemical demonstration of 11β-hydroxysteroid dehydrogenase as a marker for Leydig cell maturation in rat. *Acta Histochem., Suppl* 38, 203–207.

Haigh, R. M., and Jones, C. T. (1990). Effect of glucocorticoids on alpha 1-adrenergic receptor binding in rat vascular smooth muscle. *J. Mol. Endocrinol.* 5, 41–48.

Hammami, M. M., and Siiteri, P. K. (1990). Cortisol resistance and altered 11β-hydroxysteroid dehydrogenase activity. *Abstr. 72nd Annu. Meet. Endocr. Soc.* Abstr. 1025; p. 281.

Hammami, M. M., and Siiteri, P. K. (1991). Regulation of 11β-hydroxysteroid dehydrogenase activity in human skin fibroblasts: Enzymatic modulation of glucocorticoid action. *J. Clin. Endocrinol. Metab.* 73, 326–334.

Haning, R., Tait, S. A. S., and Tait, J. F. (1970). *In vitro* effects of ACTH, angiotensins, serotonin and potassium on steroid output and conversion of corticosterone to aldosterone by isolated adrenal cells. *Endocrinology (Baltimore)* 87, 1147–1167.

Harinck, H. I. J., Van Brummelen, P., Van Seters, A. P., and Moolenaar, A. J. (1984). Apparent mineralocorticoid excess and deficient 11β-oxidation of cortisol in a young female. *Clin. Endocrinol. (Oxford)* 21, 605–614.

Hausmann, W., and Tarnoky, A. L. (1968). Clinical and biochemical effects of carbenoxolone. *In* "A Symposium on Carbenoxolone Sodium" (J. M. Robson and F. M. Sullivan, eds.), pp. 159–172. Butterworth, London.

Hayashi, T., Nakai, T., Uchida, K., Morimoto, S., and Takeda, R. (1984). The characteristics of renal mineralocorticoid receptors in glycyrrhizic acid or deoxycorticosterone-induced hypertensive rats. *Clin. Exp. Hypertens.—Theory Pract.* A6, 1625–1640.

Hechter, O., Zaffaroni, A., Jacobsen, R. P., Levy, H., Jeanloz, R. W., Schenker, V., and Pincus, G. (1951). The nature and biogenesis of the adrenal secretory product. *Recent Prog. Horm. Res.* 6, 215–241.

Hechter, O., Solomon, M. M., and Caspi, E. (1953). Corticosteroid metabolism in liver. Studies on perfused rat livers. *Endocrinology (Baltimore)* 53, 202–216.

Helbock, H. J., and Reynolds, J. W. (1970). Pseudoaldosteronism (Liddle's syndrome): Evidence for increased cell membrane permeability to Na^+. *Pediatr. Res.* 455.

Helenius, A., and Simons, K. (1975). Solubilization of membranes by detergent. *Biochim. Biophys. Acta* 415, 29–79.

Hellman, L., Bradlow, H. L., Zumoff, B., and Gallagher, T. F. (1961). The influence of thyroid hormone on hydrocortisone production and metabolism. *J. Clin. Endocrinol. Metab.* 21, 1231–1247.

Hierholzer, K., Castello, R., Kobayashi, N., and Fromm, M. (1990a). Sites and significance of renal corticosteroid metabolism. *Int. Congr. Ser.—Excerpta Med.* 877, 67–76.

Hierholzer, K., Siebe, H., and Fromm, M. (1990b). Inhibition of 11β-hydroxysteroid dehydrogenase and its effect on epithelial sodium transport. *Kidney Int.* 38, 673–678.

Hierholzer, K., Bühler, H., and Perschel, F. H. (1991). Target cell metabolism of corticosteroids mediating antisteroid effects. *In* "Nephrology" (M. Hatano, ed.), pp. 1122–1136. Springer-Verlag, Tokyo.

Hjelmeland, L. M., and Chrambach, A. (1984). Solubilization of functional membrane-bound receptors. *In* "Receptor Biochemistry and Methodology" (J. Craig Venter and L. C. Harrison, eds.), Vol. 1, pp. 35–46. Liss, New York.

Hoff, H. G., Ghraf, R., Raible, M., and Schriefers, H. (1973). Ontogenese von Hydroxysteroid Dehydrogenase-Aktivitaten in der Ratten leber. *Hoppe-Seyler's Z. Physiol. Chem.* 354, 306–311.

Hollander, J. L., Brown, E. M., Jessar, R. A., and Brown, C. Y. (1951). Hydrocortisone and cortisone injected into arthritic joints. Comparative effects of and use of hydrocortisone as a local anti-arthritic agent. *JAMA, J. Am. Med. Assoc.* 147, 1629–1635.

Holmes, W. N., Broock, R. L., and Devlin, J. M. (1974). Tritiated corticosteroid metabolism in intact and adenohypophysectomized ducks. *Gen. Comp. Endocrinol.* 22, 417–427.

Honour, J. W., Dillon, M. J., Levin, M., and Shah, V. (1983). Fatal, low renin hypertension associated with a disturbance of cortisol metabolism. *Arch. Dis. Child.* 58, 1018–1020.

Hoyer, P. E., and Moller, M. (1977). Histochemistry of 11β-hydroxysteroid dehydrogenases in rat submandibular gland. Effect of cortisol stimulation. *Histochem. J.* 9, 599–618.

Hsia, S. L., and Hao, Y. L. (1966). Metabolic transformations of cortisol-4- [^{14}C] in human skin. *Biochemistry* 5, 1469–1474.
Hubener, H. J., Fukushima, D. K., and Gallagher, T. F. (1956). Substrate specificity of enzymes reducing the 11- and 20-keto groups of steroids. *J. Biol. Chem.* 220, 499–511.
Hughes, H., and Cowles, R. J. (1977). Estimation of plasma levels of glycyrrhetinic acid. *N. Z. Med. J.* 85, 398–405.
Hummelink, R., and Ballard, P. L. (1986). Endogenous corticoids and lung development in the fetal rabbit. *Endocrinology (Baltimore)* 118, 1622–1629.
Humphrey, M. J., Lindup, W. E., Chakraborty, J., and Parke, D. V. (1979). Effect of carbenoxolone on the concentrations of aldosterone in rat plasma and kidney. *J. Endocrinol.* 81, 143–151.
Hurlock, B., and Talalay, P. (1959). Microsomal 3α and 11β-hydroxysteroid dehydrogenases. *Arch. Biochem. Biophys.* 80, 469–470.
Ichikawa, Y. (1966). Metabolism of cortisol-4-^{14}C in patients with infectious and collagen diseases. *Metab., Clin. Exp.* 15, 613–625.
Idler, D. R., and MacNab, H. C. (1967). The biosynthesis of 11-ketotestosterone and 11β-hydroxytestosterone by Atlantic Salmon tissue in vitro. *Can. J. Biochem. Physiol.* 45, 581–589.
Idler, D. R., and Truscott, B. (1963). *In vivo* metabolism of steroid hormones by sockeye salmon. *Can. J. Biochem. Physiol.* 41, 875–887.
Idler, D. R., Ronald, A. P., and Schmidt, P. J. (1959a). Isolation of cortisone and cortisol from the plasma of Pacific salmon. *J. Am. Chem. Soc.* 81, 1260–1261.
Idler, D. R., Ronald, A. P., and Schmidt, R. J. (1959b). Biochemical studies on sockeye salmon during spawning migration. *Can. J. Biochem. Physiol.* 37, 1227–1238.
Idler, D. R., Sangalang, G. B., and Truscott, B. (1972). Corticosteroids in South American Lungfish. *Gen. Comp. Endocrinol., Suppl.* 3, 238–244.
Ingle, D. J. (1940). The effect of two cortin-like compounds upon body weight and work performance of adrenalectomized rats. *Endocrinology (Baltimore)* 27, 297–304.
Ishida, S., Ichikawa, T., and Sakiya, Y. (1988). Binding of glycyrrhetinic acid to rat plasma, rat serum albumin, human serum and human serum albumin. *Chem. Pharm. Bull.* 36, 440–443.
Itoh, K., Hara, T., Shiraishi, T., Taniguchi, K., Morimoto, S., and Onishi, T. (1989). Effects of glycyrrhizin and glycyrrhetinic acid on (Na$^+$ + K$^+$)-ATPase of renal basolateral membranes *in vitro*. *Biochem. Int.* 18, 81–89.
Jahn, G. A., Moguilewsky, M., Houdebine, L.-M., and Djiane, J. (1987). Binding and action of glucocorticoids and mineralocorticoids in rabbit mammary gland. Exclusive participation of glucocorticoid type II receptors for stimulation of casein synthesis. *Mol. Cell. Endocrinol.* 52, 205–212.
Jazayeri, A., and Meyer, W. J. (1988). Glucocorticoid modulation of β-adrenergic receptors of cultured rat arterial smooth muscle cells. *Hypertension* 12, 393–398.
Jazayeri, A., and Meyer, W. J. (1989). Mineralocorticoid induced increase in β-adrenergic receptors of cultured rat arterial smooth muscle cells. *J. Steroid Biochem.* 33, 987–991.
Jornvall, H., Persson, M., and Jeffrey, J. (1981). Alcohol and polyol dehydrogenases are both divided into two protein types, and structural properties cross-relate the different enzyme activities within each type. *Proc. Natl. Acad. Sci. U.S.A.* 78, 4226–4230.
Kanaoka, M., Yano, S., and Kato, H. (1988). Preparation of [3α-^{3}H]3β-hydroxy-18β- and 3α-hydroxy-18β and α-glycyrrhetic acid and radioimmunoassay of glycyrrhetic acid. *Chem. Pharm. Bull.* 36, 3264–3270.

Kaplan, N. O. (1968). Nature of multiple molecular forms of enzymes. *Ann N.Y. Acad. Sci.* 151, 382–399.
Katz, A. J. (1990). Corticosteroid regulation of Na-K-ATPase along the mammalian nephron. *Semin. Nephrol.* 10, 388–399.
Kendall, E. C. (1941). The function of the adrenal cortex. *JAMA, J. Am. Med. Assoc.* 116, 2394–2398.
Kime, D. E. (1978). The hepatic catabolism of cortisol in teleost fish—Adrenal origin of 11-oxotestosterone precursors. *Gen. Comp. Endocrinol.* 35, 322–328.
Kinsella, J. L. (1990). Action of glucocorticoids on proximal tubule transport systems. *Semin. Nephrol.* 10, 330–338.
Kittinger, G. W. (1974). Feto-maternal production and transfer of cortisol in the rhesus. *Steroids* 23, 229–243.
Kobayashi, N., Schulz, W., and Hierholzer, K. (1987). Corticosteroid metabolism in rat kidney in vitro. IV. Subcellular sites of 11β-hydroxysteroid dehydrogenase activity. *Pfluegers Arch.* 408, 46–53.
Koerner, D. R. (1966). 11β-hydroxysteroid dehydrogenase of lung and testis. *Endocrinology (Baltimore)* 79, 935–938.
Koerner, D. R. (1969). Assay and substrate specificity of liver 11β-hydroxysteroid dehydrogenase. *Biochim. Biophys. Acta* 176, 377–382.
Koerner, D. R., and Hellman, L. (1964). Effect of thyroxine administration on the 11β-hydroxysteroid dehydrogenases in rat liver and kidney. *Endocrinology (Baltimore)* 75, 592–601.
Kolanowski, J., Corcell-Corf, F., and Lammerant, J. (1981). cortisol uptake, release and conversion into cortisone by the heart muscle in dogs. *J. Steroid Biochem.* 14, 773–781.
Kornel, L. (1970). Corticosteroids in human blood V Extraadrenal effects of ACTH upon metabolism of cortisol. *Steroidologia* 1, 225–244.
Kornel, L. (1988). Disparate effects of glucocorticoids and mineralocorticoids on sodium and water transport in vascular smooth muscle. *Proc. 70th Annu. Meet. Endocr. Soc, New Orleans, LA* p. 99.
Kornel, L., Starnes, W. R., Hill, S. R., Jr., and Hill, A. (1969). Studies on steroid conjugates VI Quantitative paper chromatography of urinary corticosteroids in essential hypertension. *J. Clin. Endocrinol. Metab.* 29, 1608–1617.
Kornel, L., Wu, F. T., and Saito, Z. (1975). Essential hypertension: A derangement in corticosteroid metabolism. *Rush-Presbyt.-St. Luke's Med. Bull.* 14, 3–16.
Kornel, L., Kanamarlapudi, N., Travers, T., Taff, D. J., Patel, N., Chen, C., Baum, R. M., and Raynor, W. J. (1982). Studies of high affinity binding of mineralo- and glucocorticoids in rabbit aorta cytosol. *J. Steroid Biochem.* 16, 245–264.
Kraan, G. P. B., Derks, H. J. G. M., and Drayer, N. M. (1980). Quantification of polar glucocorticosteroids in the urine of pregnant and nonpregnant women: A comparison with 6α-hydroxylated metabolites of cortisol in neonatal urine and amniotic fluid. *J. Clin. Endocrinol. Metab.* 51, 754–758.
Krozowski, Z. S., and Funder, J. W. (1983). Renal mineralocorticoid receptors and hippocampal corticosterone binding species have identical intrinsic steroid specificity. *Proc. Natl. Acad. Sci. U.S.A.* 80, 6056–6060.
Krozowski, Z. S., Rundle, S. E., Wallace, C., Castell, M. J., Shen, J.-H., Dowling, J., Funder, J. W., and Smith, I. A. (1989). Immunolocalization of renal mineralocorticoid receptors with an antiserum against a peptide deduced from the complementary deoxyribonucleic acid sequence. *Endocrinology (Baltimore)* 125, 192–198.
Krozowski, Z. S., Stuchbery, S., White, P., Monder, C., and Funder, J. W. (1990). Charac-

terization of 11β-hydroxysteroid dehydrogenase gene expression: Identification of multiple unique forms of messenger ribonucleic acid in the rat kidney. *Endocrinology (Baltimore)* 127, 3009–3013.

Krozowski, Z. S., Obeyesekere, V., Smith, R., and Mercer, W. (1992). Tissue-specific expression of an 11β-hydroxysteroid dehydrogenase with a truncated N-terminal domain—a potential mechanism for differential intracellular localization within mineralocorticoid target cells. *J. Biol. Chem.* 267, 2569–2574.

Kumamoto, J., Raison, J. K., and Lyons, J. M. (1971). Temperature breaks in Arrhenius plots. A thermodynamic consequence of a phase change. *J. Theor. Biol.* 31, 47–51.

Lakshmi, V., and Monder, C. (1985a). Extraction of 11β-hydroxysteroid dehydrogenase from rat liver by detergents. *J. Steroid Biochem.* 22, 331–340.

Lakshmi, V., and Monder, C. (1985b). Evidence for independent 11-oxidase and 11-reductase activities of 11β-hydroxysteroid dehydrogenase: Enzyme latency, phase transitions and lipid requirements. *Endocrinology (Baltimore)* 116, 552–560.

Lakshmi, V., and Monder, C. (1988). Purification and characterization of the corticosteroid 11β-dehydrogenase component of the rat liver 11β-hydroxysteroid dehydrogenase complex. *Endocrinology (Baltimore)* 123, 2390–2398.

Lakshmi, V., Sakai, R. R., McEwen, B. S., and Monder, C. (1991). Regional distribution of 11β-hydroxysteroid dehydrogenase in rat brain. *Endocrinology (Baltimore)* 128, 1741–1748.

Latif, S. A., Conca, T. J., and Morris, D. J. (1990). The effects of the licorice derivative, glycyrrhetinic acid, on hepatic 3α and 3β-hydroxysteroid dehydrogenases and 5α and 5β-reductase pathway of metabolism of aldosterone in male rats. *Steroids* 55, 52–58.

Lax, E. R., Ghraf, R., and Schriefers, H. (1978). The hormonal regulation of hepatic microsomal 11β-hydroxysteroid dehydrogenase activity in the rat. *Acta Endocrinol. (Copenhagen)* 89, 352–358.

Lax, E. R., Ghraf, R., Schriefers, H., and Voigt, K. H. (1979). The involvement of the thyroid and adrenal in the regulation of enzyme activities of hepatic and renal steroid metabolism in the rat. *Hoppe-Seyler's Z. Physiol. Chem.* 360, 137–143.

Lee, S.-M. K., Chekal, M. A., and Katz, A. I. (1983). Corticosteroid binding sites along the rat nephron. *Am. J. Physiol.* 244, F504–F509.

Leitz, T., and Reinboth, R. (1987). The biosynthesis of 11-ketotestosterone by the testis of the Siamese fighting fish Betta splendens regan (Anabantoideri, Belontiidae). *Gen. Comp. Endocrinol.* 66, 145–157.

Levitz, M., Jansen, V., and Dancis, J. (1978). The transfer and metabolism of corticosteroids in the perfused human placenta. *Am. J. Obstet. Gynecol.* 132, 363–366.

Liddle, G. W., Bledsoe, T., and Coppage, W. S. (1963). A familial renal disorder simulating primary aldosteronism but with negligible aldosterone secretion. *Trans. Assoc. Am. Physicians* 76, 199–211.

Liggins, G. C. (1976). Adrenocortical-related maturational events in the fetus. *Am. J. Obstet. Gynecol.* 126, 931–939.

Liggins, G. C., and Howie, R. N. (1972). A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics* 50, 515–525.

Lugg, M. A., and Nicholas, T. E. (1978). The effect of dexamethasone on the activity of 11β-hydroxysteroid dehydrogenase in the foetal rabbit lung during the final stages of gestation. *J. Pharm. Pharmacol.* 30, 587–588.

MacKenzie, M. A., Hoefnagels, W. H. L., Jansen, R. W. M. M., Benraad, T. J., and Kloppenborg, P. W. C. (1990). The influence of glycyrrhetinic acid on plasma cortisol and cortisone in healthy young volunteers. *J. Clin. Endocrinol. Metab.* 70, 1637–1643.

Mahesh, V. B., and Ulrich, F. (1960). Metabolism of cortisol and cortisone by various tissues and subcellular particles. *J. Biol. Chem.* 235, 356–360.

Marekov, L., Krook, M., and Jornvall, H. (1990). Prokaryotic 20β-hydroxysteroid dehydrogenase is an enzyme for the 'short-chain, non-metalloenzyme' alcohol dehydrogenase type. *FEBS Lett.* 266, 51–54.

Marver, D., and Edelman, I. S. (1978). Dihydrocortisol, a potent mineralocorticoid. *J. Steroid Biochem.* 9, 1–7.

Mason, H. L. (1950). Isolation of adrenal cortical hormones from urine: 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone. *J. Biol. Chem.* 182, 131–149.

Masters, J. N., Finch, C. E., and Sapolsky, R. M. (1989). Glucocorticoid endangerment of hippocampal neurons does not involve deoxyribonucleic acid cleavage. *Endocrinology (Baltimore)* 124, 3083–3088.

Mattingly, D., Tyler, C., and Bilton, E. (1970). Plasma 11-hydroxycorticoid levels after, carbenoxolone sodium. *Br. Med. J.* 3, 498–501.

McDonald, I. R., Than, K. A., and Evans, B. (1988). Glucocorticoids in the blood plasma of the platypus Ornithorynchus anatinus. *J. Endocrinol.* 118, 407–416.

McEwen, B. S., Lambdin, L. T., Rainbow, T. C., and De Nicola, A. F. (1986). Aldosterone effects on salt appetite in adrenalectomized rats. *Neuroendocrinology* 43, 38–43.

McGinnis, J. F., and de Vellis, J. (1981). Cell surface modulation of gene expression in brain cells by down regulation of glucocorticoid receptors. *Proc. Natl. Acad. Sci. U.S.A.* 78, 1288–1292.

Meigs, R. A., and Engel, L. L. (1961). The metabolism of adrenocortical steroids by human tissues. *Endocrinology (Baltimore)* 69, 152–162.

Mercer, W. R., and Krozowski, Z. S. (1992). Localization of an 11β hydroxysteroid dehydrogenase activity to the distal nephron. Evidence for the existence of two species of dehydrogenase in the rat kidney. *Endocrinology (Baltimore)* 130, 540–543.

Meyer, W. J., and Nichols, N. R. (1981). Mineralocorticoid binding in cultured smooth muscle cells and fibroblasts from rat aorta. *J. Steroid Biochem.* 14, 1157–1168.

Michaud, N. J., and Burton, A. F. (1977). Maternal fetal relationships in corticosteroid metabolism. *Biol. Neonate* 32, 132–137.

Miller, L. L., and Axelrod, L. R. (1953). Cortisone metabolism in the perfused normal and experimental cirrhotic rat liver. *Metab. Clin. Exp.* 3, 438–448.

Milora, R., Vagnucci, A., and Goodman, A. D. (1967). A syndrome resembling primary aldosteronism but without mineralocorticoid excess. *Clin. Res.* 15, 482.

Mitchell, B. F., Seron-Ferré, M., Hess, D. L., and Jaffe, R. B. (1981). Cortisol production and metabolism in the late gestation rhesus monkey fetus. *Endocrinology (Baltimore)* 108, 916–924.

Mitchell, B. F., Seron-Ferré, M., and Jaffe, R. B. (1982). Cortisol-cortisone interrelationships in the late gestation rhesus monkey fetus *in utero*. *Endocrinology (Baltimore)* 111, 1837–1842.

Miyabo, S., Kishida, S., and Hisada, T. (1973). Metabolism and conjugation of cortisol by various dog tissues *in vitro*. *J. Steroid Biochem.* 4, 567–576.

Moguilewsky, M., and Raynaud, J. P. (1980). Evidence for a specific mineralocorticoid receptor in rat pituitary and brain. *J. Steroid Biochem.* 12, 309–314.

Moisan, M.-P., Seckl, J. R., Monder, C., Agarwal, A. K., White, P. C., and Edwards, C. R. W. (1990a). 11β-hydroxysteroid dehydrogenase in brain: mRNA expression and bioactivity in rat cerebellum. *J. Neuroendocrinol.* 2, 853–858.

Moisan, M.-P., Seckl, J. R., and Edwards, C. R. W. (1990b). 11β-hydroxysteroid dehydro-

genase bioactivity and messenger RNA expression in rat forebrain: Localization in hypothalamus, hippocampus and cortex. *Endocrinology (Baltimore)* 127, 1450–1455.

Moisin, M.-P., Edwards, C. R. W., and Seckl, J. R. (1992). Ontogeny of 11β-hydroxysteroid dehydrogenase in rat brain and kidney. *Endocrinology (Baltimore)* 130, 400–404.

Molina, R., Filella, X., Herranz, M., Prats, M., Velasco, A., Zanon, G., Martinez-Osaba, M. J., and Ballista, A. M. (1990). Biochemistry of cyst fluid in fibrocystic disease of the breast. *Ann. N.Y. Acad. Sci.* 586, 29–42.

Monder, C. (1991a). Heterogeneity of 11β-hydroxysteroid dehydrogenase in rat tissues. *J. Steroid Biochem.* 40, 533–536.

Monder, C. (1991b). Corticosteroids, receptors, and the organ-specific functions of 11β-hydroxysteroid dehydrogenase. *FASEB J.* 5, 3047–3054.

Monder, C. (1991c). Corticosteroids, kidneys, sweet roots and dirty drugs. *Mol. Cell. Endocrinol.* 78, C95–C98.

Monder, C., and Bradlow, H. L. (1980). Cortoic acids: Exploration at the frontier of corticosteroid metabolism. *Recent Prog. Horm. Res.* 36, 365–400.

Monder, C., and Lakshmi, V. (1989a). Evidence for kinetically distinct forms of corticosteroid 11β-dehydrogenase in rat liver microsomes. *J. Steroid Biochem.* 32, 77–83.

Monder, C., and Lakshmi, V. (1989b). Corticosteroid 11β-hydroxysteroid dehydrogenase activities in vertebrate liver. *Steroids* 52, 515–528.

Monder, C., and Lakshmi, V. (1990). Corticosteroid 11β-dehydrogenase of rat tissues: Immunological studies. *Endocrinology (Baltimore)* 126, 2435–2443.

Monder, C., and Shackleton, C. H. L. (1984). 11β-hydroxysteroid dehydrogenase: Fact or fancy?. *Steroids* 44, 383–417.

Monder, C., and White, A. (1963). Purification and properties of a sheep liver 21-hydroxysteroid dehydrogenase. *J. Biol. Chem.* 238, 767–774.

Monder, C., and White, A. (1965). The 21-hydroxysteroid dehydrogenases of liver: A NADP⁺ dehydrogenase and two NAD⁺ dehydrogenases. *J. Biol. Chem.* 240, 71.

Monder, C., Shackleton, C. H. L., Bradlow, H. L., New, M. I., Stoner, E., Iohan, F., and Lakshmi, V. (1986). The syndrome of apparent mineralocorticoid excess: Its association with 11β-dehydrogenase and 5β-reductase deficiency and some consequences for corticosteroid metabolism. *J. Clin. Endocrinol. Metab.* 63, 550.

Monder, C., Stewart, P. M., Lakshmi, V., Valentino, R., Burt, D., and Edwards, C. R. W. (1989). Licorice inhibits corticosteroid 11β-dehydrogenase of rat kidney and liver: In vivo and in vitro studies. *Endocrinology (Baltimore)* 125, 1046–1053.

Monder, C., Lakshmi, V., and Miroff, Y. (1991). Kinetic studies on rat liver 11β-hydroxysteroid dehydrogenase. *Biochim. Biophys. Acta* 1115, 23–29.

Monder, C., Sakai, R. R., Blanchard, R. J., Blanchard, D. C., Lakshmi, V., Miroff, Y., Phillips, D. M., and Hardy, M. (1993). The mediation of testicular function by 11β-hydroxysteroid dehydrogenase. *In* "Stress and Reproduction" (J. W. Funder and J. H. Boublick, eds.), Raven Press, New York (in press).

Moura, A.-M., and Worcel, M. (1984). Direct action of aldosterone on transmembrane ^{22}Na efflux from arterial smooth muscle. *Hypertension* 6, 425–430.

Munck, A., and Leung, K. (1977). Glucocorticoid receptors and mechanisms of action. *In* "Receptors and Mechanisms of Action of Steroid Hormones" (J. Pasqualini, ed.), pp. 311–397. Dekker, New York.

Murphy, B. E. P. (1977a). Chorionic membrane as an extra-adrenal source of foetal cortisol in human amniotic fluid. *Nature (London)* 266, 179–181.

Murphy, B. E. P. (1977b). Conversion of cortisol to cortisone by the human uterus and its reversal in pregnancy. *J. Clin. Endocrinol. Metab.* 44, 1214–1217.

Murphy, B. E. P. (1978). Cortisol production and inactivation by the human lung during gestation and infancy. *J. Clin. Endocrinol. Metab.* 47, 243–248.

Murphy, B. E. P. (1979a). The influence of serum proteins on the metabolism of cortisol by the human placenta. *J. Steroid Biochem.* 10, 387–392.

Murphy, B. E. P. (1979b). Cortisol and cortisone in human fetal development. *J. Steroid Biochem.* 11, 509–513.

Murphy, B. E. P. (1981). Ontogeny of cortisol-cortisone interconversion in human tissues: A role for cortisone in human fetal development. *J. Steroid Biochem.* 14, 811–817.

Murphy, B. E. P. (1982). The absorption by the human fetus of intra-amniotically injected cortisol. *J. Steroid Biochem.* 16, 415–417.

Murphy, B. E. P. (1991). Steroids and depression. *J. Steroid Biochem. Mol. Biol.* 38, 537–559.

Murphy, B. E. P., and Branchaud, C. T. L. (1983). Fetal metabolism of cortisol. *Curr. Top. Exp. Endocrinol.* 5, 197–229.

Murphy, B. E. P., and Diez d'Aux, R. C. (1972). Steroid levels in the human fetus: Cortisol and cortisone. *J. Clin. Endocrinol. Metab.* 35, 678–683.

Murphy, B. E. P., and Vedady, D. (1981). Specificity of human 11β-hydroxysteroid dehydrogenase. *J. Steroid Biochem.* 14, 807–809.

Murphy, B. E. P., and Vedady, D. L. (1982). Radioenzymatic assay for some substrates and inhibitors of human placental 11-hydroxysteroid dehydrogenase. *J. Immunoassay* 13, 17–30.

Murphy, B. E. P., Clark, S. J., Donald, I. R., Pinsky, M., and Vedady, D. (1974). Conversion of maternal cortisol to cortisone during placental transfer to the human fetus. *Am. J. Obstet. Gynecol.* 118, 538–541.

Naray-Fejes-Toth, A., and Fejes-Toth, G. (1990). Glucocorticoid receptors mediate mineralocorticoid-like effects in cultured collecting duct cells. *Am. J. Physiol.* 259, F672–F678.

Naray-Fejes-Toth, A., Watlington, C. O., and Fejes-Toth, G. (1991). 11β-hydroxysteroid dehydrogenase activity in the renal target cells of aldosterone. *Endocrinology (Baltimore)* 129, 17–21.

Nelson, D. H., Samuels, L. T., Willardson, D. G., and Tyler, F. H. (1951). The levels of 17-hydroxycorticosteroids in peripheral blood of human subjects. *J. Clin. Endocrinol.* 11, 1021–1029.

New, M. I., and Levine, L. S. (1977). An unidentified ACTH-stimulable adrenal steroid in childhood hypertension. *In* "Juvenile Hypertension" (M. I. New and L. S. Levine, eds.), pp. 143–163. Raven Press, New York.

New, M. I., Levine, L., Biglieri, E. G., Pareira, J., and Ulick, S. (1977). Evidence for an unidentified steroid in a child with apparent mineralocorticoid hypertension. *J. Clin. Endocrinol. Metab.* 44, 924–933.

New, M. I., Oberfield, S. E., Carey, R., Grieg, F., Ulick, S., and Levine, L. S. (1982). A genetic defect in cortisol metabolism as the basis for the syndrome of apparent mineralocorticoid excess. *Serono Symp.* 50, 85–101.

Nguyen-Trong-Tuan, Rekdal, D. J., and Burton, A. F. (1971). The uptake and metabolism of ^{3}H-corticosterone and fluorimetrically determined corticosterone in fetuses of several mouse strains. *Biol. Neonate* 18, 78–84.

Nicholas, T. E., and Kim, P. A. (1975). The metabolism of ^{3}H cortisone- and ^{3}H-cortisol by the isolated perfused rat and guinea pig lungs. *Steroids* 25, 387–402.

Nicholas, T. E., and Lugg, M. A. (1982). The physiological significance of 11β-hydroxysteroid dehydrogenase in the rat lung. *J. Steroid Biochem.* **17**, 113–118.

Nichols, N. R., Olsson, C. A., and Funder, J. W. (1983). Steroid effects on protein synthesis in cultured smooth muscle cells from rat aorta. *Endocrinology (Baltimore)* **113**, 1096–1101.

Nichols, N. R., McNally, M., Campbell, J. H., and Funder, J. W. (1984). Overlapping but not identical protein synthetic domains in cardiovascular cells in response to glucocorticoid hormones. *J. Hypertens.* **2**, 663–669.

Nichols, N. R., Nguyen, H. H., and Meyer, W. J., III (1986). Physical separation of aortic corticoid receptors with Type I and Type II specificities. *J. Steroid Biochem.* **22**, 577–582.

Noma, J., Hayashi, N., and Sekiba, K. (1991). Automated direct HPLC assay for estetrol, estriol, cortisone and cortisol in serum and amniotic fluid. *J. Chromatogr.* **568**, 35–44.

Oberfield, S. E., Levine, L. S., Carey, R. M., Greig, F., Ulick, S., and New, M. I. (1983). Metabolic and blood pressure responses to hydrocortisone in the syndrome of apparent mineralocorticoid excess. *J. Clin. Endocrinol. Metab.* **56**, 332–339.

Ojima, M., Satoh, K., Gomibuchi, T., Itoh, N., Kim, S., Fukuchi, S., and Miyachi, Y. (1990). The inhibitory effects of glycyrrhizin and glycyrrhetinic acid on the metabolism of cortisol and prednisolone. *Nippon Naibunpi Gakkai Zasshi* **66**, 584–596.

Olson, R. E., Thayer, S. A., and Kopp, L. J. (1944). The glycogenic activity of certain crystalline steroids of the adrenal cortex when administered singly and with cortical extract to fasted, normal, and adrenalectomized rats. *Endocrinology (Baltimore)* **35**, 464–472.

Ong, J., Kerr, D. I. B., Capper, H. R., and Johnston, G. A. R. (1990). Cortisone: A potent $GABA_A$ antagonist in the guinea pig isolated ileum. *J. Pharm. Pharmacol.* **42**, 662–664.

Onoyama, K., Bravo, E. L., and Tarazi, R. C. (1979). Sodium, extracellular fluid volume and cardiac output changes in the genesis of mineralocorticoid hypertension in the intact dog. *Hypertension* **1**, 331–336.

Osinski, P. A. (1960). Steroid 11β-dehydrogenase in human placenta. *Nature (London)* **187**, 777.

Pal, S. B. (1967). Pattern of the excretion of urinary cortisol, tetrahydrocortisone, allotetrahydrocortisol and tetrahydrocortisol in normal human individuals and in patients with rheumatic disorders. *Clin. Chim. Acta* **15**, 57–67.

Pasqualini, J. R., Nguyen, B.-L., Uhlrich, F., Wiquist, N., and Diczfalusy, E. (1970a). Cortisol and cortisone metabolism in the human feto-placental unit at midgestation. *J. Steroid Biochem.* **1**, 209–219.

Pasqualini, J. R., Costa-Novaes, S. C., Ito, Y., and Nguyen, B.-L. (1970b). Reciprocal cortisol-cortisone conversions in the total tissue and subcellular fractions of fetal and adult guinea pig liver. *J. Steroid Biochem.* **1**, 341–347.

Pepe, G. J. (1979). The production and secretion of cortisol by baboon neonates. *Steroids* **33**, 251–260.

Pepe, G. J., and Albrecht, E. D. (1984a). Comparison of cortisol-cortisone interconversion *in vitro* by the human and baboon placenta. *Steroids* **44**, 229–239.

Pepe, G. J., and Albrecht, E. D. (1984b). Transuteroplacental metabolism of cortisol and cortisone during mid- and late gestation in the baboon. *Endocrinology (Baltimore)* **115**, 1946–1951.

Pepe, G. J., and Albrecht, E. D. (1985a). The effects of cortisone on the interconversion of cortisol and cortisone in the baboon. *J. Steroid Biochem.* **23**, 275–278.

Pepe, G. J., and Albrecht, E. D. (1985b). Transplacental corticosteroid metabolism during baboon pregnancy. *In* "Research in Perinatal Medicine. IV. Perinatal Endocrinology" (E. D. Albrecht and G. D. Pepe, eds.), p. 201. Perinatology Press, New York.

Pepe, G. J., and Albrecht, E. D. (1987). Fetal regulation of transplacental cortisol-cortisone metabolism in the baboon. *Endocrinology (Baltimore)* **120**, 2529–2533.

Pepe, G. J., and Townsley, J. D. (1976). The metabolism of cortisol by term baboon neonates (*Papio papio*). *Endocrinology (Baltimore)* **99**, 466–469.

Pepe, G. J., Waddell, B. J., Stahl, S. J., and Albrecht, E. D. (1988). The regulation of transplacental cortisol-cortisone metabolism by estrogen in pregnant baboons. *Endocrinology (Baltimore)* **122**, 78–83.

Perschel, F. H., Bühler, H., and Hierholzer, K. (1991). Bile acids and their amidates inhibit 11β-hydroxysteroid dehydrogenase obtained from rat kidney. *Pfluegers Arch.* **418**, 538–543.

Peterson, N. A., Chaikoff, I. L., and Jones, C. (1965). The *in vitro* conversion of cortisol to cortisone by subcellular brain fractions of young and adult rats. *J. Neurochem.* **12**, 273–278.

Peterson, R. E., and Pierce, C. E. (1960). The metabolism of corticosterone in man. *J. Clin. Invest.* **39**, 741–757.

Peterson, R. E., Wyngaarden, J. B., Guerra, S. L., Brodie, B. B., and Bunim, J. J. (1955). The physiological disposition and metabolic fate of hydrocortisone in man. *J. Clin. Invest.* **34**, 1779.

Phillipou, G., and Higgins, B. A. (1985). A new defect in the peripheral conversion of cortisone to cortisol. *J. Steroid Biochem.* **22**, 435–436.

Phillips, D. M., Lakshmi, V., and Monder, C. (1989). Corticosteroid 11β-dehydrogenase in rat testis. *Endocrinology (Baltimore)* **125**, 209–216.

Pinder, R. M., Brogden, R. N., Sawyer, P. R., Speight, T. M., Spencer, R., and Avery, G. S. (1976). Carbenoxolone: A review of its pharmacological properties and therapeutic efficacy in peptic ulcer disease. *Drugs* **11**, 245–307.

Porter, G. A. (1970). Synergistic effect of carbenoxolone sodium on aldosterone-enhanced active sodium transport in toad skin. *In* "A Symposium on Carbenoxolone Sodium" (J. H. Baron and F. M. Sullivan, eds.), pp. 33–47. Butterworth, London.

Quirk, S. J., Gannell, J. E., and Funder, J. W. (1983). Aldosterone binding sites in pregnant and lactating rat mammary glands. *Endocrinology (Baltimore)* **113**, 1812–1817.

Quirk, S. J., Slattery, J., and Funder, J. W. (1990a). 11β-hydroxysteroid dehydrogenase activity in the mammary gland. *J. Steroid Biochem.* **35**, 623–625.

Quirk, S. J., Slattery, J. A., and Funder, J. W. (1990b). Epithelial and adipose cells isolated from mammary glands of pregnant and lactating rats differ in 11β-hydroxysteroid dehydrogenase activity. *J. Steroid Biochem. Mol. Biol.* **37**, 529–534.

Raison, J. K., Lyons, J. M., Mehlhorn, R. J., and Keith, A. D. (1971). Temperature-induced phase changes in mitochondrial membranes detected by spin labeling. *J. Biol. Chem.* **246**, 4036–4040.

Rajin, S. (1972). Reconstitution of biological membranes. *Biochim. Biophys. Acta* **256**, 241–296.

Ramirez, L. C., Levine, L. S., Gunczler, P., Zanconato, G., Ramirez, L. C., Rauh, W., Rosler, A., Bradlow, H. L., and New, M. I. (1979). A syndrome of mineralocorticoid excess associated with defects in the peripheral metabolism of cortisol. *J. Clin. Endocrinol. Metab.* **49**, 757–764.

Reevers, F. (1948). Behandeling van uleus ventriculi in uleus duodeni met succus liqueritiae. *Ned. Tijdschr. Geneeskd.* 92, 2968–2971.

Reich, H., Nelson, D. H., and Zaffaroni, A. (1950). Isolation of 17-hydroxycorticosterone from blood obtained from adrenal veins of dogs. *J. Biol. Chem.* 187, 411–417.

Reichstein, T., and Shoppee, C. W. (1943). The hormones of the adrenal cortex. *Vitam. Horm. (N.Y.)* 1, 345–413.

Reul, J. M. H. M., and de Kloet, E. R. (1985). Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology (Baltimore)* 117, 2505–2511.

Rogoff, J. M., and Stewart, G. N. (1927). The influence of adrenal extracts on the survival period of adrenalectomized dogs. *Science* 66, 327–328.

Romanoff, L. P., Rodriguez, R. M., Seelye, J. M., and Pincus, G. (1957). Determination of tetrahydrocortisol and tetrahydrocortisone in the urine of normal and schizophrenic men. *J. Clin. Endocrinol. Metab.* 17, 777–785.

Rosenblum, P. M., Yamada, L., Callard, I. P., and Callard, G. V. (1985). Validation of radioimmunoassay systems for the measurement of 11-keto- and 11β-hydroxytestosterone in teleost blood. *Comp. Biochem. Physiol. B* 82B, 659–665.

Rousseau, G. G., Baxter, J. D., and Tomkins, G. M. (1972). Glucocorticoid receptors: Relations between steroid binding and biological effects. *J. Mol. Biol.* 67, 99–115.

Rousseau, R., Baxter, J. D., Funder, J. W., Edelman, I. S., and Tomkins, G. M. (1972). Glucocorticoid and mineralocorticoid receptors for aldosterone. *J. Steroid Biochem.* 3, 219–227.

Rundle, S. E., Funder, J. W., Lakshmi, V., and Monder, C. (1989a). The intrarenal localization of mineralocorticoid receptors and 11β-dehydrogenase: Immunocytochemical studies. *Endocrinology (Baltimore)* 125, 1700–1704.

Rundle, S. E., Smith, I. A., Stockman, D., and Funder, J. W. (1989b). Immunocytochemical demonstration of mineralocorticoid receptors in rat and human kidney. *J. Steroid Biochem.* 33, 1235–1242.

Sakai, R. R., Lakshmi, V., Monder, C., Funder, J. W., Krozowski, Z., and McEwen, B. S. (1990). Colocalization of 11β-hydroxysteroid dehydrogenase and type I receptor immunoreactivity in the rat brain. *Soc. Neurosci. Abstr.* 16, Part 2, 1309.

Sakai, R. R., Jellinck, H. L., and Monder, C. (1993). In preparation.

Sandor, T., and Mehdi, A. Z. (1980). Corticosteroids and their role in the extrarenal electrolyte secreting organs of nonmammalian vertebrates. *In* "Steroids and Their Mechanism of Action in Nonmammalian Vertebrates" (G. Delrio and J. Brachet, eds.), pp. 33–49. Raven Press, New York.

Sandor, T., Mehdi, A. Z., and Fazekas, A. G. (1977). Corticosteroid binding macromolecules in the salt activated nasal gland of the domestic duck Anas platyrhynchos. *Gen. Comp. Endocrinol.* 32, 348–359.

Sandor, T., Mehdi, A. Z., and DiBattista, J. A. (1983). Further studies on the corticosteroid-receptor system of the nasal gland of the domestic duck (Anas platyrrhynchos). *Can. J. Biochem. Cell Biol.* 61, 731–743.

Sang, G. W., Lorenzo, B., and Reidenberg, M. M. (1991). Inhibitory effects of gossypol on corticosteroid 11β-hydroxysteroid dehydrogenase from guinea pig kidney: A possible mechanism for causing hypokalemia. *J. Steroid Biochem. Mol. Biol.* 39, 169–176.

Sapolsky, R. M., and Pulsinelli, W. A. (1985). Glucocorticoids potentiate ischemic injury to neurons: Therapeutic implications. *Science* 229, 1381–1397.

Savage, D. C. L., Forsyth, C. C., McCafferty, E., and Cameron, J. (1975) The excretion of

individual adrenocortical steroids during normal childhood and adolescence. *Acta Endocrinol (Copenhagen)* 79, 551–567.

Savard, K., Burstein, S., Rosenkrantz, H., and Dorfman, R. I. (1953). The metabolism of adrenosterone in vivo. *J. Biol. Chem.* 202, 717–725.

Schulz, W., Kobayashi, N., Siebe, H., and Hierholzer, K. (1987). 11β-hydroxysteroid dehydrogenase (11-HSD)—its function in renal corticosteroid metabolism. *In* "Molecular Nephology: Biochemical Aspects of Kidney Function" (Z. Kovacevic and W. G. Guder, eds.), pp. 361–367. de Gruyter, Berlin.

Schulze, H. U., and Speth, M. (1980). Investigations on the possible involvement of phospholipids in the glucose-6-phosphate transport system of rat liver microsomal glucose-6-phosphatase. *Eur. J. Biochem.* 106, 505–514.

Seckl, J. R., Kelly, P.A.T., and Sharkey, J. (1991). Glycyrrhetinic acid, an inhibitor of 11β-hydroxysteroid dehydrogenase, alters local cerebral glucose utilization *in vivo*. *J. Steroid Biochem. Mol. Biol.* 39, 777–779.

Bennett, J. A., Brown, R. D., Inland, D. P., Yorbro, L. R., Watson, J. T., Slaton, P. E., Hollifield, H. W., and Liddle, G. W. (1976). Evidence for a new mineralocorticoid in patients with low-renin essential hypertension. *Circ. Res.* 36, Suppl. 1, 2–9.

Shackleton, C. H. L., Honour, J. W., Dillon, M. J., Chantler, C., and Jones, R. W. A. (1980). Hypertension in a 4 year old child-GC/MS evidence for deficient hepatic metabolism of steroids. *J Clin. Endocrinol. Metab.* 50, 786–792.

Shackleton, C. H. L., Rodriguez, J., Arteaga, E., Lopez, J. M., and Winter, J. S. D. (1985). Congenital 11β-hydroxysteroid dehydrogenase deficiency associated with juvenile hypertension: Corticosteroid metabolite profiles of four patients and their families. *Clin. Endocrinol. (Oxford)* 22, 701–712.

Sheppard, K., and Funder, J. W. (1987a). Mineralocorticoid specificity of renal type I receptors. *Am. J. Physiol.* 252, E224–E229.

Sheppard, K., and Funder, J. W. (1987b). Type I receptors in parotid, colon, and pituitary are aldosterone selective *in vivo*. *Am. J. Physiol.* 253, E467–E471.

Shollon, L. J., Work, E. E., Jr., and MacGee, J. (1965). Metabolism of cortisol-4-^{14}C and cortisone-4-^{14}C by rat brain homogenates. *Metab. Clin. Exp.* 14, 1122–1127.

Slikker, W., Jr., Althaus, Z. R., Rowland, J. M., Hill, D. E., and Hendrickx, A. G. (1982). Comparison of the transplacental pharmacokinetics of cortisol and triamcinolone acetonide in the rhesus monkey. *J. Pharmacol. Exp. Ther.* 223, 368–374.

Sloviter, R. S., Valiquette, G., and Abrams, G. M. (1989). Selective loss of hippocampal granule cells in the mature rat brain after adrenalectomy. *Science* 243, 535–538.

Smith, B. T. (1978). The role of pulmonary corticosteroid 11-reductase activity in lung maturation in the fetal rat. *Pediatr. Res.* 12, 12–14.

Smith, B. T., Torday, J. S., and Giroud, C. J. P. (1973). The growth promoting effect of cortisol on human fetal lung cells. *Steroids* 22, 515–524.

Smith, B. T., Taeswell, K. A., Minshall, D., Bogues, W. N., and Vreeken, E. (1982). Influence of corticosteroids on glycogen content and steroid 11-reductase activity in lung and liver of the fetal and newborn rat. *Biol. Neonate* 42, 201–207.

Smith, R. E., and Funder, J. W. (1991). Renal 11β-hydroxysteroid dehydrogenase activity: Effects of age, sex, and altered hormonal status. *J. Steroid Biochem. Mol. Biol.* 38, 265–267.

Souness, G. W., and Morris, D. J. (1990). The effects of 11-dehydrocorticosterone (A) on the Na$^+$ retaining actions of aldosterone. *Program, 72nd Annu. Meet. Endocr. Soc. Abstr.* 486, p. 146.

Souness, G. W., and Morris, D. J. (1991). 11-dehydrocorticosterone (compound A) in the presence of carbenoxolone is a more potent sodium retainer than its parent steroid

corticosterone (compound B). *Program 73rd Annu. Meet. Endocr. Soc.* Abstr. 664, p. 196.
Sowell, J. G., Hagen, A. A., and Troop, R. C. (1971). Metabolism of cortisone-4-^{14}C by rat lung tissue. *Steroids* 18, 289–301.
Sprague, R. G., Mason, H. L., and Power, M. H. (1951). Physiologic effects of cortisone and ACTH in man. *Recent Prog. Horm. Res.* 6, 315–365.
Steiger, M., and Reichstein, T. (1937). Desoxycorticosterone (21-oxyprogesterone) aus 5-3-oxydatio-kohlensauer. *Helv. Chim. Acta* 20, 1164–1179.
Stetten, M. R., and Burnett, F. F. (1967). Some properties of variously activated microsomal glucose-6-phosphatase, inorganic phosphatase and inorganic pyrophosphate-glucose phosphotransferase: Shift in pH optimum. *Biochim. Biophys. Acta* 139, 138–147.
Stewart, P. M., and Edwards, C. R. W. (1990). Specificity of the mineralocorticoid receptor: Crucial role of 11β-hydroxysteroid dehydrogenase. *TEM* 1, 225–230.
Stewart, P. M., and Edwards, C. R. W. (1991). The cortisol-cortisone shuttle and hypertension. *J. Steroid Biochem. Mol. Biol.* 40, 501–509.
Stewart, P. M., Wallace, A. M., Valentino, R., Burt, D., Shackleton, C. H. L., and Edwards, C. R. W. (1987). Mineralocorticoid activity of liquorice: 11β-hydroxysteroid dehydrogenase deficiency comes of age. *Lancet* 1, 821–823.
Stewart, P. M., Corrie, J. E. T., Shackleton, C. H. L., and Edwards, C. R. W. (1988). Syndrome of apparent mineralocorticoid excess. A defect in the cortisol-cortisone shuttle. *J. Clin. Invest.* 82, 340–349.
Stewart, P. M., Whorwood, C. B., Barber, P., Gregory, J., Monder, C., Franklyn, J. A., and Sheppard, M. C. (1991). Localization of renal 11β-dehydrogenase by in situ hybridization: Autocrine not paracrine protector of the mineralocorticoid receptor. *Endocrinology (Baltimore)* 128, 2129–2135.
Suhara, K., Takeda, K., and Katagiri, M. (1986). P450$_{11\beta}$-dependent conversion of cortisol to cortisone, and 19-hydroxyandrostenedione to 19-oxoandrostenedione. *Biochem. Biophys. Res. Commun.* 136, 369–375.
Sweat, M. L., and Bryson, M. J. (1960). Role of phosphopyridine nucleotides in the metabolism of cortisol by peripheral muscle. *Biochim. Biophys. Acta* 44, 217–223.
Swingle, W. W., and Remington, J. W. (1944). The role of the adrenal cortex in physiological processes. *Physiol. Rev.* 24, 89–127.
Takeda, R., Miyamori, I., Soma, R., Matsubara, T., and Ikeda, M. (1987). Glycyrrhizic acid and its hydrolysate as mineralocorticoid agonist. *J. Steroid Biochem.* 27, 845–849.
Takemoto, D. J., Abel, J. H., Jr., and Allen, J. C. (1976). The metabolism of corticosterone in the salt gland of the duck, *Anas platyrhynchos*. *Gen. Comp. Endocrinol.* 28, 226–232.
Tamura, Y., Nishikawa, T., Yamada, K., Yamamoto, M., and Kumagai, A. (1979). Effects of glycyrrhetinic acid and its derivatives on delta-4-5 alpha and 5 β reductase in rat liver. *Arzneim.-Forsch.* 29, 647–649.
Tanford, C., and Reynolds, J. A. (1976). Characterization of membrane proteins in detergent solutions. *Biochim. Biophys. Acta* 457, 133–170.
Tannin, G. M., Agarwal, A. K., Monder, C., New, M. I., and White, P. C. (1991). The human gene for 11β-hydroxysteroid dehydrogenase: Structure, tissue distribution and chromosomal localization. *J. Biol. Chem.* 266, 16653–16658.
Tanswell, A. K., Worthington, D., and Smith, B. T. (1977). Human Amniotic membrane corticosteroid 11-oxidoreductase activity. *J. Clin. Endocrinol. Metab.* 45, 721–725.
Tashima, Y., Terui, M., Stoh, H., Mizunuma, H., Kobayashi, R., and Marumo, F. (1989). Identical properties of aldosterone and corticosterone binders and their presence in rat brain and kidney. *J. Biochem. (Tokyo)* 106, 446–454.
Taylor, N. F., Bartlett, W. A., Dawson, D. J., and Enoch, B. A. (1984). Cortisone reductase deficiency: Evidence for a new inborn error of metabolism of adrenal steroids. *J. Endocrinol., Suppl* 102 Abstr. 50.
Teelucksingh, S., Mackie, A. D. R., Burt, D., McIntyre, M. A., Brett, L., and Edwards, C. R. W. (1990). Potentiation of hydrocortisone activity in skin by glycyrrhetinic acid. *Lancet* 1, 1060–1063.
Tempel, D. L., and Liebowitz, S. F. (1989). PVN steroid implants: Effect on feeding patterns and macronutrient selection. *Brain Res. Bull.* 23, 553–560.
Thorn, G. W. (1944). Clinical use of extracts from adrenal cortex. *JAMA, J. Am. Med. Assoc.* 125, 10–15.
Tomkins, G. M., and Isselbacher, K. J. (1954). Enzymatic reduction of cortisone. *J. Am. Chem. Soc.* 76, 3100–3101.
Torday, J. S., Smith, B. T., and Giroud, C. J. P. (1975). The rabbit fetal lung as a glucocorticoid target tissue. *Endocrinology (Baltimore)* 96, 1462–1467.
Torday, J. S. (1980). Glucocorticoid dependence of fetal lung maturation in vitro. *Endocrinology (Baltimore)* 107, 839–844.
Torday, J. S., Olson, E. B., Jr., and First, N. L. (1976). Production of cortisol from cortisone by the isolated perfused fetal rabbit lung. *Steroids* 27, 869–880.
Touchstone, J. C., Griffin, J. F., and Kasparow, M. (1963). Cortisol from human nerve. *Science* 141, 1275–1276.
Touitou, Y., Auzeby, A., Bodgan, A., Luton, J. P., and Galan, P. (1984). 11-hydroxy-11-ketosteroids equilibrium. A source of misinterpretation in steroid synthesis: evidence through the effects of trilostane on 11-hydroxysteroid dehydrogenase in sheep and human adrenals in vitro. *J. Steroid Biochem.* 20, 763–768.
Truscott, B. (1979). Steroid metabolism in fish. Identification of steroid moieties of hydrolyzable conjugates of cortisol in the bile of trout *Salmo gairdnerii*. *Gen. Comp. Endocrinol.* 38, 196–206.
Tye, L. M., and Burton, A. F. (1980). Variations in the pattern of metabolism of corticosteroids in fetal mouse tissues. *Life Sci.* 26, 35–39.
Ulick, S., Ramirez, L. C., and New, M. I. (1977). An abnormality in steroid reductive metabolism in a hypertensive syndrome. *J. Clin. Endocrinol. Metab.* 44, 799–802.
Ulick, S., Levine, L. S., Gunczler, P., Zanconato, G., Ramirez, L. C., Rauh, W., Rosler, A., and Bradlow, H. L. (1979). A syndrome of apparent mineralocorticoid excess associated with defects in the peripheral metabolism of cortisol. *J. Clin. Endocrinol. Metab.* 49, 757–764.
Ulick, S., Chan, C. K., Rao, K. N., Edassery, J., and Mantero, F. (1989). A new form of the syndrome of apparent mineralocorticoid excess. *J. Steroid Biochem.* 32, 209–212.
Ulick, S., Tedde, R., and Mantero, F. (1990). Pathogenesis of the type 2 variant of the syndrome of apparent mineralocorticoid excess. *J. Clin. Endocrinol. Metab.* 70, 200–206.
Ullmann, A., Menard, J., and Corvol, P. (1975). Binding of glycyrrhetinic acid to kidney mineralocorticoid receptors. *Endocrinology (Baltimore)* 97, 46–51.
Unger, F., Gunville, R., and Seabloom, R. W. (1978). Seasonal variation in adrenal 11β-hydroxysteroid dehydrogenase in the meadow vole. *Gen. Comp. Endocrinol.* 36, 111–118.
Van den Berg, D. T. W. M., deKloet, E. R., Van Dijken, H. H., and DeJong, W. (1990). Differential central effects of mineralocorticoid and glucocorticoid agonist and antagonist on blood pressure. *Endocrinology (Baltimore)* 126, 118–124.

NCBI National Library of Medicine PubMed

PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM

Search PubMed for Go Clear

Limits Preview/Index History Clipboard

Display Abstract Save Text Order Add to Clipboard

Entrez PubMed

PubMed Services

Related Resources

☐1: *Endocr Res* 1996 Nov;22(4):793-801 Related Articles, Books, LinkOut

Endogenous 11 beta-hydroxysteroid dehydrogenase inhibitors and their role in glucocorticoid Na+ retention and hypertension.

Morris DJ, Souness GW

Department of Pathology and Laboratory Medicine, Miriam Hospital, Lifespan and Brown University School of Medicine, Providence, RI 02906, USA.

11 beta-hydroxysteroid dehydrogenase (11 beta-HSD) metabolizes active glucocorticoids to their inactive 11-dehydro products and protects renal mineralocorticoid receptors from the high circulating levels of endogenous glucocorticoids. 11 beta-HSD has been suggested to be important not only in the control of renal sodium retention but also blood pressure. We had previously shown that 11 alpha- and 11 beta-hydroxyprogesterone (11 alpha- and 11 beta-OHP) were (I) potent inhibitors of 11 beta-HSD (Isoforms 1 and 2) activity in vitro, (ii) able to confer mineralocorticoid (MC) activity upon corticosterone (B) in vivo and (iii) hypertensinogenic when chronically infused into Sprague-Dawley (SD) rats. In addition we also showed that 3 alpha,5B-tetrahydroprogesterone (3 alpha,5B-THP) and chenodeoxycholic acid (CDCA) were potent inhibitors of 11 beta-HSD1 activity but not 11 beta-HSD2 activity, however, these substances were still able to confer MC activity upon B in the adrenalectomized rat. To assess the possible blood pressure modulating effects of 3 alpha,5B-THP and CDCA we have now infused these substances into intact SD rats continuously for 14 days. Both 3 alpha,5B-THP and CDCA caused a significant elevation in blood pressure within seven days, an effect that persisted throughout the 14-day infusion. These results show that both 3 alpha,5B-THP and CDCA are hypertensinogenic in the rat and that the inhibition of either 11 beta-HSD2 or 11 beta-HSD1 activity by endogenous progesterone metabolites and CDCA may be involved in the pathology of hypertension.

PMID: 8969942, UI: 97124806

Display Abstract Save Text Order Add to Clipboard

Write to the Help Desk

1: J Clin Endocrinol Metab. 1995 Nov;80(11):3155-9. Related Articles, Links

Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation.

Walker BR, Connacher AA, Lindsay RM, Webb DJ, Edwards CR.

University of Edinburgh, Department of Medicine, Western General Hospital, Scotland.

In the kidney, conversion of cortisol to cortisone by the enzyme 11 beta-hydroxysteroid dehydrogenase protects mineralocorticoid receptors from cortisol. In the liver, a different isoform of the enzyme favors 11 beta-reductase conversion of cortisone to cortisol. We have tested the hypothesis that hepatic 11 beta-reductase enhances glucocorticoid receptor activation in the liver by inhibiting the enzyme with carbenoxolone and observing effects on insulin sensitivity. Seven healthy males took part in a double blind randomized cross-over study in which oral carbenoxolone (100 mg every 8 h) or placebo was administered for 7 days. Euglycemic hyperinsulinemic clamp studies were then performed, including measurement of forearm glucose uptake. Carbenoxolone increased whole body insulin sensitivity (M values for dextrose infusion rates, 41.1 +/- 2.4 mumol/kg.min for placebo vs. 44.6 +/- 2.3 for carbenoxolone; $P < 0.03$), but had no effect on forearm insulin sensitivity. We infer that carbenoxolone, by inhibiting hepatic 11 beta-reductase and reducing intrahepatic cortisol concentration, increases hepatic insulin sensitivity and decreases glucose production. Thus, plasma cortisone provides an inactive pool that can be converted to active glucocorticoids at sites where 11 beta-reductase is expressed, abnormal hepatic 11 beta-reductase activity might be important in syndromes of insulin resistance, and manipulation of hepatic 11 beta-reductase may be useful in treating insulin resistance.

Publication Types:
- Clinical Trial
- Randomized Controlled Trial

MeSH Terms:
- 11-beta-Hydroxysteroid Dehydrogenases
- Adult
- Blood Glucose/metabolism
- Carbenoxolone/pharmacology*
- Cross-Over Studies
- Double-Blind Method
- Forearm/blood supply

- Human
- Hydroxysteroid Dehydrogenases/physiology*
- Insulin/blood
- Insulin/physiology*
- Liver/drug effects*
- Liver/physiology*
- Male
- Receptors, Glucocorticoid/physiology*
- Support, Non-U.S. Gov't

Substances:

- Blood Glucose
- Receptors, Glucocorticoid
- Insulin
- Carbenoxolone
- Hydroxysteroid Dehydrogenases
- 11-beta-Hydroxysteroid Dehydrogenases

PMID: 7593419 [PubMed - indexed for MEDLINE]

Display Citation Show: 20 Sort Send to Text

1: *Endocrinology* 1993 Jun;132(6):2287-92 Related Articles, Books

Licorice inhibits 11 beta-hydroxysteroid dehydrogenase messenger ribonucleic acid levels and potentiates glucocorticoid hormone action.

Whorwood CB, Sheppard MC, Stewart PM

Department of Medicine, University of Birmingham, Queen Elizabeth Hospital, Edgbaston, United Kingdom.

11 beta-Hydroxysteroid dehydrogenase (11 beta HSD) is responsible for the interconversion of cortisol to cortisone [corticosterone (B) to 11-dehydrocorticosterone in rodents] and confers ligand specificity to the mineralocorticoid receptor. Inhibition of 11 beta HSD by licorice derivatives [glycyrrhizic and glycyrrhetinic (GE) acids] results in cortisol/B and not aldosterone acting as a potent mineralocorticoid. 11 beta HSD is ubiquitously expressed and, by converting active glucocorticoid to inactive metabolites, may be an important prereceptor regulator of ligand access to the glucocorticoid receptor (GR). To investigate this further, we have studied the effect of 11 beta HSD inhibition by licorice derivatives on PRL gene expression (a known glucocorticoid target gene) in rat pituitary GH3 cells. Glycyrrhizic acid administration to rats in vivo (75 mg/kg.day for 5 days) resulted in inhibition of 11 beta HSD activity, as previously reported, but also a significant reduction in steady state 11 beta HSD mRNA levels in both predominantly mineralocorticoid (kidney and distal colon) and glucocorticoid (liver and pituitary) target tissues. In vitro, 11 beta HSD mRNA and activity were present in rat pituitary GH3 cells (81% conversion of B to 11-dehydrocorticosterone/4 x 10(6) cells after 24-h incubation) and inhibited by GE in a dose-dependent fashion. While B or GE alone (10(-8)-10(-5) M) had little or no effect on PRL mRNA levels or immunoassayable PRL, combinations of GE plus B resulted in marked inhibition of PRL mRNA levels and secretion, to such an extent that a concentration of 10(-6) M B with 10(-6) M GE was more potent than equimolar concentration of the synthetic GR agonist RU 28362. This inhibitory effect on PRL mRNA levels was blocked by a 10-fold excess of the GR antagonist RU 38486, but not by a 10-fold excess of the mineralocorticoid receptor antagonist RU 26752, confirming that this potentiation of glucocorticoid hormone action was operating through the GR and not the mineralocorticoid receptor. In addition to its established role as a competitive inhibitor of 11 beta HSD, licorice results in pretranslational inhibition of 11 beta HSD both in vitro and in vivo. 11 beta HSD is clearly an important mechanism in regulating tissue

levels of active glucocorticoid and, hence, ligand supply to the GR.

PMID: 8504732, UI: 93279206

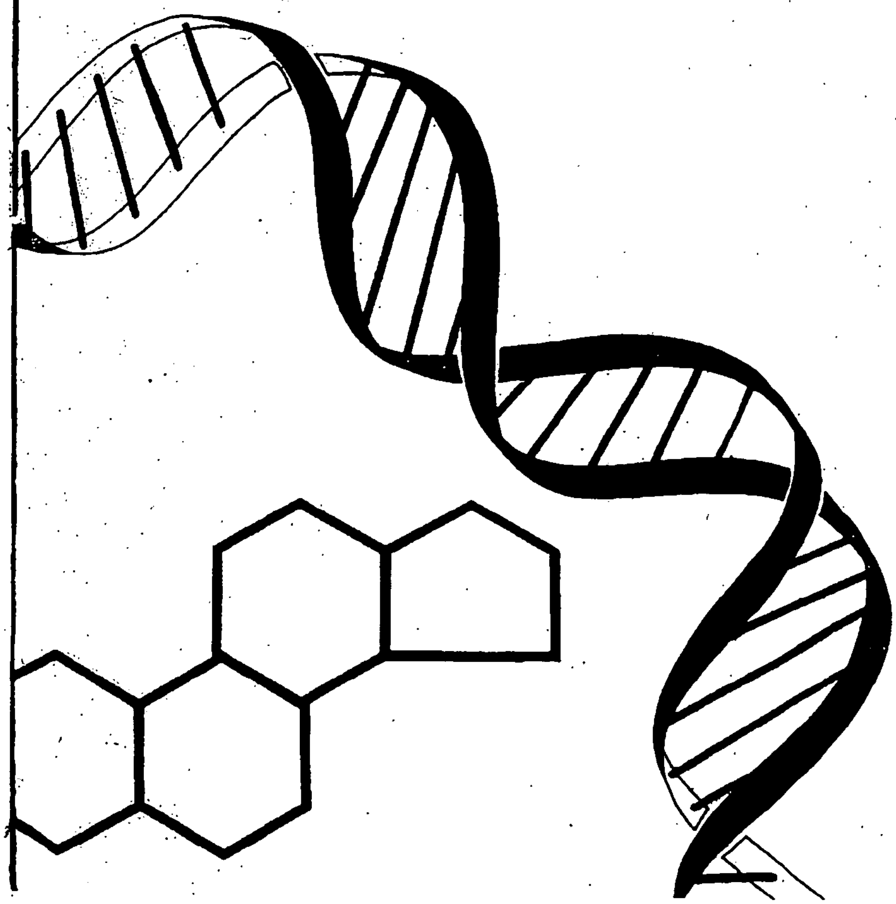
Display Abstract Save Text Order Add to Clipboard

ISSN 0960-0760

Volume 49 Number 1

May 1994

The Journal of Steroid Biochemistry & Molecular Biology



PERGAMON

Pergamon

0960-0760(94)E0010-I

J. Steroid Biochem. Molec. Biol. Vol. 49, No. 1, pp. 81–85, 1994

0960-0760/94 $7.00 + 0.00

Inhibition of 11β-Hydroxysteroid Dehydrogenase Obtained from Guinea Pig Kidney by Furosemide, Naringenin and Some Other Compounds

Yin Di Zhang,* Beverly Lorenzo and Marcus M. Reidenberg†

Departments of Pharmacology and Medicine, Cornell University Medical College, 1300 York Avenue, New York, NY 10021, U.S.A.

Inhibition of 11β-hydroxysteroid dehydrogenase (11β-OHSD) can cause excess mineralocorticoid effects and hypokalemia. Several substances causing hypokalemia (glycyrrhizic acid in licorice and gossypol) inhibit this enzyme. We tested other compounds for activity to inhibit 11β-OHSD in guinea pig kidney cortex microsomes with NADP as cofactor and cortisol as substrate. Furosemide was an inhibitor while bumetanide was not, indicating a mechanism for the increased K^+ excretion caus d by furosemide compared with bumetanide. Naringenin (found in grapefruit juice), ethacrynic acid, and chenodeoxycholic acid had inhibitor IC_{50} values similar to glycyrrhizic acid. We conclude that various compounds can inhibit this enzyme and may play a role in K^+ metabolism and adrenocorticosteroid action.

J. Steroid Biochem. Molec. Biol., Vol. 49, No. 1, pp. 81–85, 1994

INTRODUCTION

The syndrome of apparent mineralocorticoid excess, first described by Ulick, Ramirez and New in 1977 [1], has led to much research on the enzyme 11β-hydroxysteroid dehydrogenase (11β-OHSD). Deficient activity of this enzyme in children leads to their inability to oxidize cortisol to inactive cortisone, providing high cortisol levels in the kidney which activate renal mineralocorticoid receptors and cause hypertension and hypokalemia. Subsequently, the mechanism of licorice-induced hypermineralocorticoidism was shown to be the inhibition of 11β-OHSD by the active principle of licorice, glycyrrhizic acid. Since then, much research has been done to explore the role that this enzyme plays in regulating the interactions of cortisol with mineralocorticoid and glucocorticoid receptors [2–6].

Gossypol, a polyphenolic constituent of cotton seed, has been studied in China as a potential oral contraceptive for men because it suppresses sperm motility and formation without affecting testosterone levels [7]. Some Chinese men who received gossypol developed hypokalemia although the cause remained obscure [7]. This is particularly remarkable since idiopathic hypokalemia, often associated with hyperthyroidism, occurs widely in China; in addition, normal Chinese men have serum potassium levels lower than men in four other countries, with 9% having values below 3.5 mmol/l [8].

In studies investigating how gossypol causes hypokalemia, we found that gossypol inhibited 11β-OHSD activity in guinea pig [9] and human [10] renal cortical microsomes. We also found that certain bioflavonoids inhibit rat liver 11β-OHSD [10]. Others have reported inhibition of the rat kidney enzyme by bile acids [11] and by steroidal and triterpenoid compounds [12], and inhibition of the rat liver enzyme by some substances in human urine [13]. We therefore decided to test a variety of compounds for their possible enzyme inhibiting effect, choosing drugs that can cause hypokalemia or sodium retention as a side effect, flavonoids from grapefruit juice that inhibit the oxidation of dehydropyridine calcium channel blocking drugs [15–17] or sterols in vegetable oils at concentrations of 100–500 mg/dl [14].

*Present address: Nanjing Medical College, Add, 140, Han Zhong Road, Nanjing, Jiangsu, China

†Correspondence to M. M. Reidenberg, Department of Pharmacology.

Received 8 July 1993; accepted 4 Jan. 1994.

MATERIALS AND METHODS

Chemicals and solutions

Sitosterol was a gift from Eli Lilly and Co. (Indianapolis, IN). Campesterol was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were purchased from Sigma Co. (St Louis, MO).

Most sterols, furosemide, ethacrynic acid, naringin and naringenin were dissolved in ethanol and diluted with methanol. Cholic acid, chenodeoxycholic acid, bumetanide, hydrochlorothiazide and spironolactone were dissolved in methanol. Phenylbutazone and indomethacin were dissolved in distilled water (pH 9). Glycyrrhizic acid was dissolved in distilled water. Corticosterone and hydrocortisone were dissolved in methanol (144 μmol/l) and kept at -4°C.

Enzyme preparation and measurement of 11β-OHSD activity

Kidney cortex was obtained from long-haired male Hartley guinea pigs. Tissue was homogenized in Krebs–Henseleit buffer as described previously [9], except for the use of a Tekmar Tissuemizer (Cincinnati, OH). Microsomes were prepared as described previously [9], except that they were diluted to a concentration of 1.25 mg protein/ml prior to storage at -70°C.

The enzyme activity in guinea pig kidney cortex microsomes was determined by measuring the rate of conversion of cortisol to cortisone. Five minutes before incubation, 2 μl of concentrated Triton DF-18 was added to each milliliter of the microsome suspension. The assay mixture contained 500 μl Krebs–Henseleit buffer (pH 7.2), 50 μl 5 mmol/l NADP, 40 μl of 144 μmol/l phosphate–sucrose buffer, 20–50 μl (25–63.5 μg) of microsome suspension in 0.01 M phosphate-sucrose buffer and various concentrations of each compound studied. This mixture was incubated in duplicate or triplicate. The total volume was 700 μl. Methanol concentration was kept at $<$ 10%. Control studies showed that this concentration did not inhibit the reaction. After 1 h of incubation at 37°C, the reaction was terminated by the addition of 3 ml methylene chloride and 20 μl 144 μmol/l corticosterone solution as the internal standard for assay of cortisone and cortisol.

The enzyme inhibition constant for furosemide was determined by adding furosemide in various amounts to achieve concentrations from 3.9 to 62 μmol/l in the incubation mixture and cortisol concentrations of 4, 8, and 16 μmol/l. The constants were obtained from a Dixon plot and a kinetic program (Chou J, Chou T-C: Michaelis-Menton analysis with microcomputers, Disk No. 1, Elsevier-Biosoft, 1989, Cambridge, England.

A modification of the HPLC method of Sang [9] was used to measure cortisol, cortisone and corticosterone in the micr somal incubation mixture. The steroids were extracted into methylene chloride by vortexing for 1 min, then centrifuged at 750 *g* for 15 min. The aqueous layer was removed by aspiration. 300 μl of 0.1 NaOH was added to the organic phase followed by vortexing for 30 s. The mixture was centrifuged and the aqueous layer removed. The organic phase was washed with 500 μl of milli-Q water (Millipore Corp., Bedford, MA). The 1.5 ml organic phase was transferred to clean glass tubes and dried by evaporation in a 45–50°C water bath. The residue was dissolved into 200 μl of methanol and 5 μl of this solution was injected into the HPLC apparatus. A standard curve for cortisol and cortisone was determined in duplicate in each enzyme experiment by using the same amount of microsome suspension after boiling to inactivate the enzyme. Standard curves were plotted as the ratio of peak height of cortisone (or cortisol) divided by the peak height of the internal standard vs steroid concentration. All unknown concentrations of cortisol and cortisone were determined from the standard curves from each experiment. The drug concentrations that inhibited the enzyme by 50% (IC_{50}) were estimated from at least 3 different concentrations of each compound evaluated by a dose–response program (Chou and Chou: Dose-effect analysis with microcomputers, Disk No. 2, Elsevier-Biosoft. 1989, Cambridge, England).

The HPLC apparatus used for quantitating the steroids consisted of a Waters Model 6000 A solvent delivery system, U6K injector, model 680 automated gradient controller, Waters 486 tunable absorbance detector and a BBC chart recorder (Model SE 120). The mobile phase contained methanol–water (30:70, v/v) at a flow rate of 1.0 ml/min. The Waters stainless steel Novapak C_{18} column (3.9 × 150 mm, 4μ) was kept at room temperature. The retention times for cortisone, cortisol and corticosterone were 6.5, 7.0 and 9.0 min, respectively.

RESULTS

The efficacy of the compounds tested to inhibit the NADP-utilizing form of 11β-OHSD from guinea pig renal cortex with cortisol as substrate is shown in Tables 1 and 2. Furosemide was the most potent inhibitor tested, with glycyrrhizic acid, naringenin, ethacrynic acid and chenodeoxycholic acid having potencies similar to each other but an order of magnitude less potent than furosemide. Data for glycyrrhizic acid, naringenin and naringin are shown in Fig. 1. The correlation coefficient (*r* value) for the computed values agreeing with the measured values for the potent inhibitors was 0.99 for furosemide, glycyrrhizic acid, and naringenin, 0.96 for ethacrynic acid and 0.86 for chenodeoxycholic acid. It was above 0.95 for all of the other compounds tested except for phenylbutazone which was 0.86.

The observations of enzyme inhibition by furosemide at varying concentrations of cortisol is

Table 1. Inhibition of 11β-OHSD by various compounds

Compound	IC_{50} (μmol/l)	Concentrations tested (μmol/l)
Furosemide	59	12, 50, 100, 200, 500, 1000
Glycyrrhizic acid	254	132, 246, 529
Naringenin	336	12, 25, 50, 100, 1000, 2000, 5000
Ethacrynic acid	452	50, 100,200, 400, 2000
Chenodeoxycholic acid	513	200, 400, 600, 800
Phenylbutazone	1358	167, 667, 1344
Sitosterol	1395	500, 1000, 1500
Stigmasterol	1968	500, 1000, 1500
Naringin	2373	582, 1163, 1744
Cholic acid	3529	1250, 2500, 3750, 5000

Campesterol inhibited 33% at the highest concentration tested of 1000 μmol/l. Since a second higher point could not be measured because of limited solubility of the compound, an IC_{50} was not calculated.

shown as a double reciprocal plot in Fig. 2. Most of the lines converge near the ordinate. A Dixon plot indicated that the inhibition by furosemide is competitive. The enzyme kinetic constants were: $K_m = 8\ \mu$mol/l and $V_{max} = 30$ nmol/μg microsomal protein/h. The K_i for furosemide was 7.7 μmol/l nearly the same as the K_m for cortisol.

DISCUSSION

We have tested a number of compounds for their ability to inhibit the NADP-utilizing form of 11β-OHSD from guinea pig renal cortex with cortisol as substrate. We found that furosemide is a much more potent inhibitor than glycyrrhizic acid, and that naringenin, ethacrynic acid and chenodeoxycholic acid inhibit with a potency almost equal to that of glycyrrhizic acid.

The compounds selected for study were chosen for a variety of reasons: the diuretics because they cause potassium loss with spironolactone as a control since it does not; glycyrrhizic acid and the bile salts as reference compounds, since data about these compounds have been published and therefore they can be used in this study to evaluate relative potency of the other compounds studied; naringin and naringenin because they are active compounds in grapefruit juice that inhibit a particular pathway of drug oxidation (cytochrome P_{450} 3A4) and we were curious to see if they also inhibited this oxidation pathway (11β-OHSD); the sterols since they are present in vegetable oils and have a structure suggesting that they might inhibit 11β-OHSD; and the cyclooxygenase inhibitors because they inhibit prostaglandin formation and cause salt retention.

The K_m of our enzyme preparation for cortisol (8 μmol/l) is similar to that of rat for corticosterone (2 μmol/l) found by Monder *et al.* [18]. Working with purified enzyme from rat liver (gift from Dr C. Monder), we have found an IC_{50} of 12 nmol/l for glycyrrhetinic acid [10], similar to the dissociation constant of the enzyme–inhibitor complex of 8 nmol/l reported by Monder *et al.* [18]. In a previous study from our laboratory, glycyrrhizic acid had an IC_{50} of 1994 μmol/l for guinea pig renal cortex microsomes with corticosterone as the substrate without Triton in the incubation mixture [9] compared with 254 μmol/l in the present study using Triton and cortisol as the substrate. Buhler *et al.* [12] working with rat kidney microsomes and corticosterone at 0.1 μmol/l, found an IC_{50} of 4 μM; in our study of guinea pig microsomes with a substrate concentration of 23 μmol/l we found an IC_{50} of 254 μM. Perschel *et al.* [11] working with rat kidney microsomes found cholic acid to inhibit this

Table 2. Compounds that failed to inhibit 11β-OHSD

Compound	Maximum concentration tested (μmol/l)
Bumetanide	2000
Hydrochlorothiazide	8000
Indomethacin	1100
Spironolactone	2000

The maximum concentration tested was limited by the solubility of the compound.

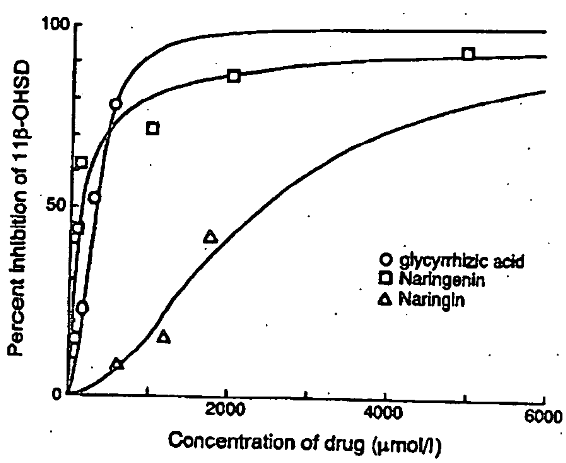


Fig. 1. Inhibition of 11[bt]β-OHSD by glycyrrhizic acid from licorice and flavonoids from grapefruit juice.

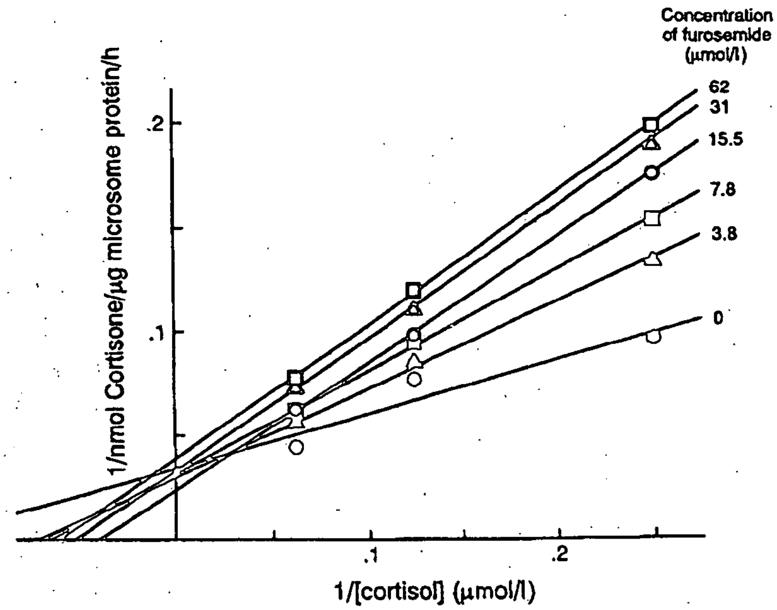


Fig. 2. Double reciprocal plot of 1/v 1/s for 11β-OHSD with varying concentrations of furosemide in incubation mixture. K_m for cortisol is 8 μmol/l. V_{max} is 30 nmol/μg microsomal protein/h. K_i for furosemide is 7.7 μmol/l.

enzyme at 1/27th the potency of chenodeoxycholic acid. We found it to be 1/7th the potency in our system.

We studied the NADP-requiring form of the enzyme that is present in most tissues rather than the NAD-requiring form that is present in the distal nephron [19–21]. Whether inhibition of the NAD-requiring enzyme is different from that of the NADP-requiring enzyme is not known. Since the mechanism of inhibition of glycyrrhetinic acid [18], gossypol [9] and furosemide (this study) is competitive, one might speculate that competitive inhibition of the NAD-requiring form of the enzyme by these compounds might also occur.

The fact that furosemide is an inhibitor of the enzyme while bumetanide is not may explain why furosemide causes more potassium excretion per unit sodium excretion than bumetanide [22–24]. It is excreted by patients with heart failure at a rate of 15–30 μg/min [25]. Assuming a 1 ml/min urine flow, the furosemide concentration would be 76 μM, compared with its IC_{50} of 59 μM in this study.

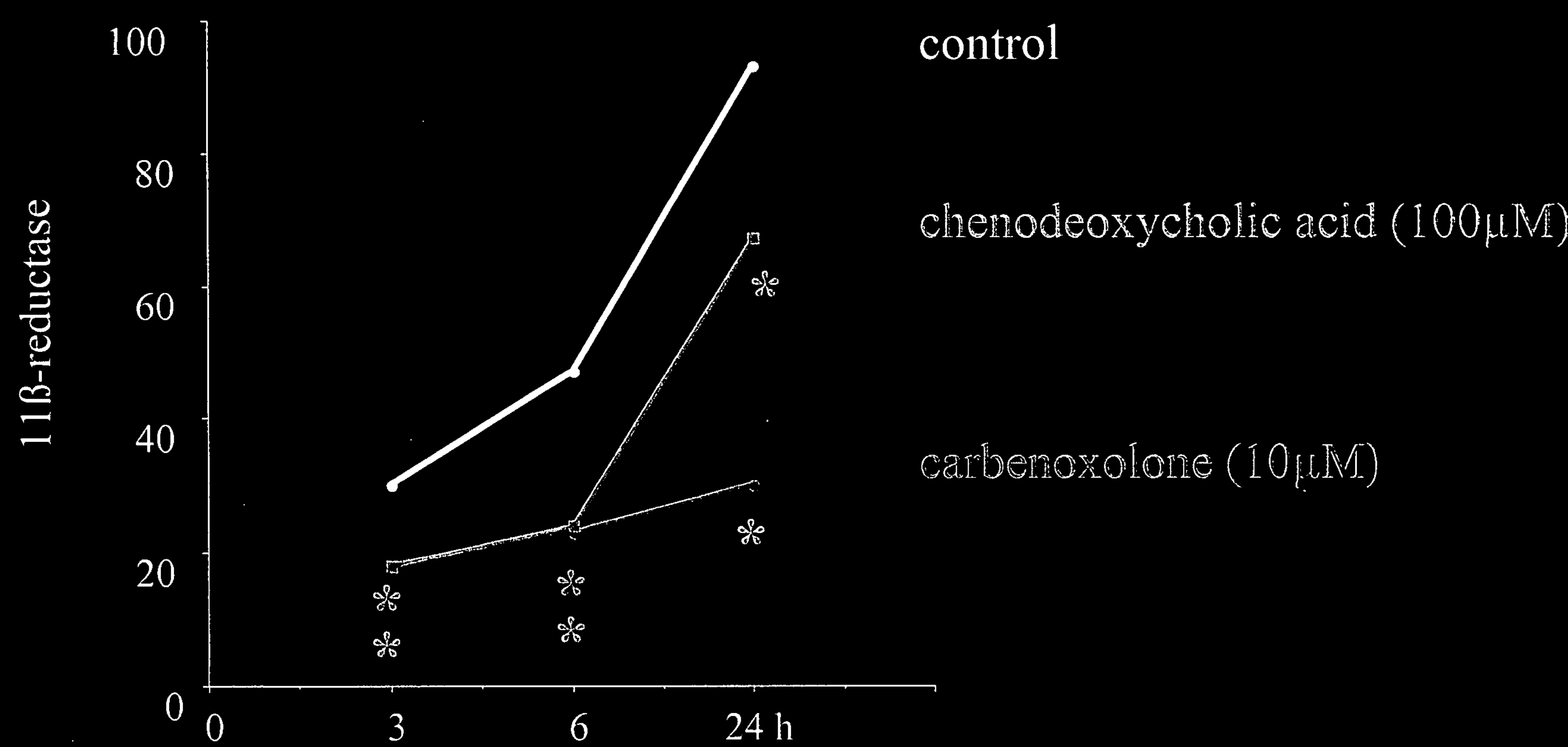
Three flavonoids: the sugar conjugates of naringenin, quercetin and kaempferol, along with some others are found in grapefruit juice [26]. These are hydrolyzed in the intestine to the aglycons which are absorbed. We found that naringenin inhibited the enzyme in this study, and previously that the flavonoids morin and quercetin were weak inhibitors [10]. The importance, if any, of these dietary constituents as *in vivo* inhibitors of this enzyme remains to be determined.

REFERENCES

1. Ulick S., Ramirez L. C. and New M. I.: An abnormality in steroid reductive metabolism in hypertensive syndrome. *J. Clin. Endocr. Metab.* 44 (1977) 799–802.
2. Monder C.: Corticosteroids, receptors, and the organ-specific functions of 11 β-hydroxysteroid dehydrogenase. *FASEB J.* 5 (1991) 3047–3054.
3. Funder J. W., Pearce P. T., Myles K. and Roy L. P.: Apparent mineralocorticoid excess, pseudohypoaldosteronism, and urinary electrolyte excretion: toward a redefinition of mineralocorticoid action. *FASEB J.* 4 (1990) 3234–3238.
4. Monder C. and White P. C.: 11 beta-hydroxysteroid dehydrogenase. *Vit. Horm.* 47 (1993) 187–271.
5. Edwards C. R. W., Walker B. R., Benediktsson R. and Seckl J. R.: Congenital and acquired syndromes of apparent mineralocorticoid excess. *J. Steroid Biochem. Molec. Biol.* 45 (1993) 1–5.
6. Edwards C. R. W.: Lessons from licorice. *New Engl. J. Med.* 325 (1991) 1242–1243.
7. Qian S. Z. and Wang Z.G.: Gossypol: a potential antifertility agent for males. *A. Rev. Pharmac. Toxic.* 24 (1984) 329–360.
8. Reidenberg M. M., Gu Z-P., Lorenzo B., Coutinho E., Athayde C., Frick J., Alvarez F., Brache V. and Emuveyan E. E.: Differences in serum potassium concentrations in normal men in different geographic locations. *Clin. Chem.* 39 (1993) 72–75.
9. Sang G. W., Lorenzo B. and Reidenberg M. M.: Inhibitory effects of gossypol on corticosteroid 11β-hydroxysteroid dehydrogenase from guinea pig kidney: a possible mechanism for causing hypokalemia. *J. Steroid Biochem. Molec. Biol.* 39 (1991) 169–176.
10. Song D., Lorenzo B. and Reidenberg M. M.: Inhibition of 11β-hydroxysteroid dehydrogenase by gossypol and bioflavonoids. *J. Lab. Clin. Med.* 120 (1992) 792–797.
11. Perschel F. H., Buhler H. and Hierholzer K.: Bile acids and their amidates inhibit 11β-hydroxysteroid dehydrogenase obtained from rat kidney. *Pflüger Archs* 418 (1991) 538–543.
12. Buhler H., Perschel F. H. and Hierholzer K.: Inhibition of rat renal 11β-hydroxysterold dehydrogenase by steroidal compounds and triterpenoids; structure/function relationship. *Biochim. Biophys. Acta* 1075 (1991) 206–212.
13. Morris D. J., Semafuko W. E. B., Latif S. A., Vogel B., Grimes C. A. and Sheff M. F.: Detection of glycyrrhetinic acid-like factors (GALFs) in human urine. *Hypertension* 20 (1992) 356–360.

14. Weihrauch J. L. and Gardner J. M.: Sterol content of foods of plant origin. *J. Am. Diet. Assoc.* 73 (1978) 39–47.
15. Bailey D. G., Spence J. D., Munoz C. and Arnold J. M. O.: Interaction of citrus juices with felodipine and nifedipine. *Lancet* 337 (1991) 268–269.
16. Miniscalco A., Lundahl J., Regardh C. G., Edgar B. and Eriksson U. G.: Inhibition of dihydropyridine metabolism in rat and human liver microsomes by flavonoids found in grapefruit in juice. *J. Pharmac.* 261 (1992) 1195–1199.
17. Soons P. A., Vogels B. A. P. M., Roosemalen M. C. M., Schoemaker H. C., Uchida E., Edgar B., Lundahl J., Cohen A. F. and Breimer D. D.: Grapefruit juice and cimetidine inhibit stereoselective metabolism of nitrendipine in humans. *Clin. Pharmac. Ther.* 50 (1991) 394–403.
18. Monder C., Stewart P. M., Lakshmi V., Valentino R., Burt D. and Edwards C. R. W.: Licorice inhibits corticosteroid 11β-dehydrogenase of rat kidney and liver: *in vivo* and *in vitro* studies. *Endocrinology* 125 (1989) 1046–1053.
19. Mercer W. R. and Krozowski Z. S.: Localization of an 11βhydroxysteroid dehydrogenase activity to the distal nephron. Evidence for the existence of two species of dehydrogenase in the rat kidney. *Endocrinology* 130 (1992) 540–543.
20. Walker B. R., Campbell J. C., Williams B. C. and Edwards C. R. W.: Tissue-specific distribution of NAD^+-dependent isoform of 11β-hydroxysteroid dehydrogenase. *Endocrinology* 131 (1992) 970–972.
21. Monder C.: The forms and functions of 11β-hydroxysteroid dehydrogenase. *J. Steroid Biochem. Molec. Biol.* 45 (1993) 161–165.
22. Asbury M. J., Gatenby P. B. B., O'Sullivan S. and Bourke E.: Bumetanide: potent new "loop" diuretic. *Br. Med. J.* 1 (1972) 211–213.
23. Ramsay L. E., McInnes G. T., Hettiarachchi J., Shelton J. and Scott P.: Bumetanide and frusemide: a comparison of dose-response curves in healthy men. *Br. J. Clin Pharmac.* 5 (1978) 243–247.
24. Brater D. C., Fox B. S. and Chennavasin P.: Electrolyte excretion patterns, intravenous and oral doses of bumetanide compared to furosemide. *J Clin. Pharmac* 21 (1981) 599–603.
25. Brater D. C., Day B., Burdette A. and Anderson S.: Bumetanide and furosemide in heart failure. *Kidney Int.* 26 (1984) 183–189.
26. Kuhnau J.: The flavonoids. A class of semi-essential food components: Their role in human nutrition. *Wld Rev. Nutr. Diet.* 24 (1976) 117–191.

Chenodeoxycholic acid inhibits 11ß-reductase in intact adipocytes



Following teachings in the application, we have identified further inhibitors of the reductase activity of 11ß-HSD1 in adipose and neuronal tissue.

Some known inhibitors of 11ß-HSD also inhibit 11ß-reductase in intact primary neurons